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BETTER THINGS FOR BETTER LIVING

. . . *THROUGH CHEMISTRY*

NECK ROT PHASE OF THE BOTRYTIS DISEASE OF GLADIOLUS[1]

J. G. Bald

Some of the main ecological features of the disease of gladiolus caused by *Botrytis gladiolorum* Timmermans may be explained by variations in the physical environment, and the reactions to it of both pathogen and host plant (5). One phase of the disease that has not previously been studied from this point of view is the Botrytis neck rot, common in southern California during autumn, winter, and spring.

Neck rot is a more or less complete rotting of the sheathing leaf bases, and sometimes also of the enclosed developing flower stem. Botrytis lesions may occur anywhere between the parent corm and a level slightly above the surface of the soil (Fig. 1, A, B), or the whole of this region may be involved[2]. The lesions are of the "aggressive" type (8) i.e., persistently capable of expansion; in this they are unlike the smaller leaf spots, which may be permanently prevented from extending by a period of exposure to unfavorable environmental conditions (7). Neck rot lesions may be relatively superficial, penetrating only the outer sheathing leaf bases, or they may penetrate the full thickness of the shoot (Fig. 1, B). Vascular bundles leading downwards from the lesions may be discolored, and the discoloration may often be traced into the bundles of the newly developing corm.

Above, the outer leaves begin to yellow and die. During continuously wet weather the bulk of the tissues at soil level may be killed, and the plant fall over and die (Fig. 1, B); if wet conditions are intermittent, the shoot may be killed but remain upright. Leaf tissues underground appear shredded and break off easily at the junction with the corm (Fig. 1, C). Under conditions of temperature and humidity favorable for Botrytis (5), sporulation occurs on the dead tissue of lesions at soil level, and may extend several inches above soil level on living shoots or on shoots that are recently dead (Fig. 1, D). Characteristic sclerotia are generally produced below ground on the more extensive lesions, and may remain attached to the dead tissue until it disintegrates (Fig. 1, C). The sclerotia may also sometimes develop on the leaf scales of the corm (5).

ORIGIN OF NECK ROT LESIONS.—Evidence was obtained that Botrytis neck rot lesions may develop in the field from:

(a) Infections on tissue below ground arising from infested corms (Fig. 1, A center). The connection between Botrytis inoculum on the corms and the incidence of neck rot was established by field observations and field and greenhouse trials. In one experiment neck rot was produced on plants from corms dug from a Botrytis-infested field. The corms were sprouted in boxes of moist sterilized soil held in constant temperature cabinets at 55°F. Healthy control corms both in sterilized soil and Botrytis-infested field soil produced plants with no sign of infection.

(b) Neck rot lesions may also develop from the downward extension of lesions produced above ground by infections from air-borne spores (Fig. 1, A right). *Botrytis gladiolorum* can grow rapidly downwards in the tissues (2) from lesions in the region of the shoot where the sheathing leaf bases begin to separate. Neck rot lesions originating in this way have the same characteristics as neck rot lesions originating from infested corms, except that there is often a greater expanse of dead tissue on which Botrytis spores may develop.

After flowers are harvested, even in the less susceptible varieties of gladiolus, Botrytis readily infects the cut surface of flower stalks or adjacent injured leaves, and grows downwards. At or below ground-level the mycelium may grow through the sheathing leaf bases, and neck rot may result. The mycelium may penetrate the corm, causing slight browning of the tissues or definite lesions under the insertion of the flower stalk. Lesions may extend along the paths of vascular bundles in the corm, at first around the core, then into it. The complete rotting and hollowing of the core so characteristic of Botrytis corm rot may be a later result of such infection (2).

Theoretically, Botrytis neck rot might begin in 2 other ways: (c) from soil-borne inoculum, and (d) from air-borne spores washed down to soil level or between the neck of the plant and surrounding soil. Field observation suggests little infection of gladiolus plants from the soil (method c), although this may not be true for regions other than southern California (6). Instances in which neck rot apparently began from direct infection by air-borne spores (d) are mentioned below.

COMPARISON OF NECK ROT AND LEAF SPOT LESIONS.—The original differentiation between aggressive and non-aggressive lesions caused by *Botrytis* spp. on *Vicia faba* (8) applies to Botrytis disease of gladiolus, as well as to Botrytis diseases of similar crops, e.g., tulip fire, due to *B. tulipae* (Lib.) Lind (7). Lesions of the aggressive type, e.g., characteristic neck rot lesions on gladiolus, are large and contain central areas of dead tissue from which the advancing margins of the lesions spread into the surrounding tissue. Abundant sporulation may occur from the dead tissue, which is filled with mycelium. *B. gladiolorum* is readily obtained in culture both from the dead centers and the advancing margins of neck-rot lesions, and of similar aggressive lesions above-ground.

[1] Accepted for publication November 26, 1952.

[2] According to botanical definition (4) the neck of the gladiolus plant is the constriction between the parent and daughter corm; the word is given a wider meaning in phytopathological literature (e.g., 3).

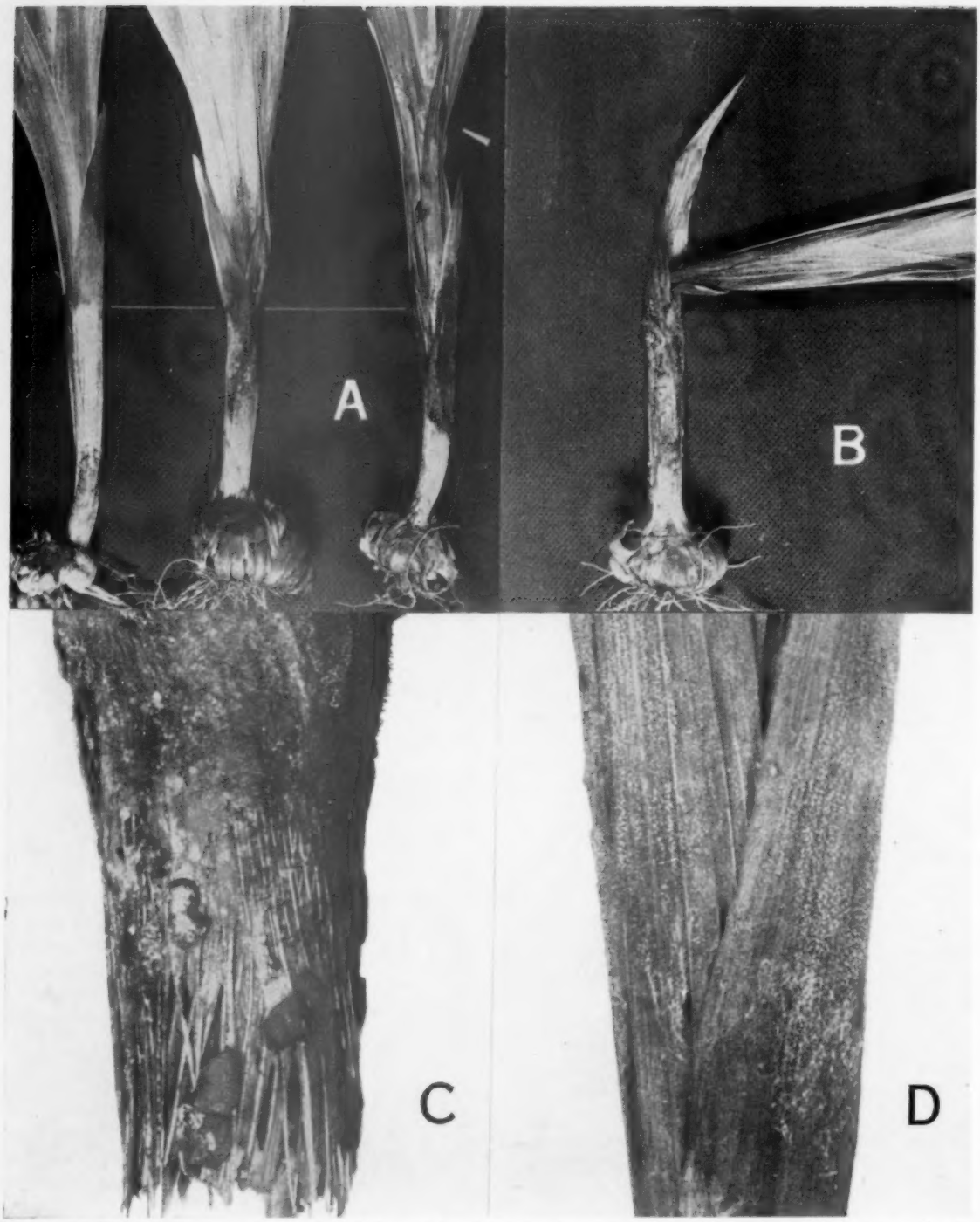

FIG. 1. A) Right, Botrytis neck rot of gladiolus arising from the downward internal extension of a lesion at the junction of the sheathing leaves indicated by pointer. Center, neck rot lesion arising underground, probably from infestation of the primordia of basal sheathing leaves or infestation of the emerging shoot. Left, healthy check plant. Soil level indicated by white line. B) Shoot rotted through by Botrytis near soil level, and fallen over. C) Last stage of Botrytis neck rot on shoot below ground. Leaf bases rotted and shredded, and detached from corm; typical sclerotia of *B. gladiolorum* present. D) Sporulation of Botrytis on a neck rot lesion extending above ground to the divergence of the sheathing leaves.

Small non-aggressive leaf spots are initially capable of extension during continuously cool moist weather; but after they have been subjected to periods of warm dry weather the majority of them neither extend

nor sporulate when again placed in a saturated atmosphere at temperatures near the optimum for the growth of Botrytis on leaf tissue. The proportion of small spots yielding *B. gladiolorum* in culture is correspondingly variable. Pieces of leaf tissue containing single small lesions, when taken from fresh infections during a Botrytis epiphytotic, have yielded up to 100 per cent colonies of *B. gladiolorum*. Collected after periods of warm dry weather, small leaf spots similarly surface sterilized and plated have yielded few colonies of *B. gladiolorum*, and sometimes none at all.

Sporulation from Neck Rot Lesions.—Many gladiolus shoots bearing dormant neck rot lesions have been placed in a saturated atmosphere at 55°–60°F. Generally within 24 or 48 hours they sporulated copiously. In the field, after 24 or 48 hours of cool wet weather, similar lesions bore abundant crops of spores.

From approximate counts and estimates it was calculated that a moderately active lesion sporulating at ground level and extending ½ to 1 in. above ground bore from ½ to 1 million spores at 1 time. This figure rose to several millions if the lesion was large and active (Fig. 1, D). The same neck rot lesion may bear successive crops of spores. During an epiphytotic, a gladiolus planting can be continuously blanketed with a spore load of many millions per acre. When an epiphytotic has reached such proportions, the most efficient spray program may be futile.

Humidity, Temperature, and the Development of Botrytis Neck Rot.—(a) *Development of neck rot in Los Angeles County*, 1949.—In southern California the neck rot phase of Botrytis is generally part of the symptom complex when leaf spotting occurs in epiphytotic proportions. Although neck rot and leaf spotting do not always occur together, the conditions favoring neck rot are essentially the same as those that encourage Botrytis leaf spot, except that they exist at and below soil level rather than above it. These appear at a mean temperature of 55°–65°F. with high humidity (5).

These conditions were first identified in the following instance. Successive plantings of gladiolus, including a number of varieties, were made throughout the early winter months of 1949 on a farm near Torrance, California, in a heavy adobe soil. By the middle of February the neck rot phase of Botrytis disease was well established. An increasing incidence of neck rot during the second half of January, February, and the first week of March was associated with high soil moisture. An abundance of Botrytis spores was produced on the neck rot lesions, the only apparent source of spores for the production of new neck rot infections. Except for a short period in February the gladiolus foliage did not remain wet long enough to start an epiphytotic of leaf spotting.

After the first week in March and until the middle of May the neck rot lesions produced relatively few spores and invaded little fresh tissue. Lack of moisture in the surface soil did not seem sufficient explanation for such slight activity of the pathogen during the whole period. A study was therefore made of soil temperature as a factor in the development of Botrytis neck rot.

(b) *Occurrence of favorable soil temperatures.*—There was no continuous record of soil temperatures at the site of this crop, except for 1 week early in March. Surface soil temperatures were found to be similar to temperatures in the same type of soil at the University campus, West Los Angeles, where records were taken over several short periods. During those periods when the weather was cool and intermittently cloudy or wet, mean temperatures throughout the surface 3 in. were about 6.5°F. above the daily mean air temperatures. According to estimates of soil temperature based on this differential, during January, February, and March, temperatures in the surface soil were mostly near or within the optimum range for development of *Botrytis gladiolorum* on the leaf tissues of gladiolus plants, that is, 55°–65°F. There were many periods when neck rot lesions developed and sporulated actively. During most of April and May, when the lesions were relatively quiescent, surface soil temperatures were within the upper range, 70°F. and over, within which Botrytis does not grow actively nor sporulate on gladiolus leaf tissue.

During much of March, soil temperatures were favorable for the development of Botrytis, but there was little activity of the fungus after the first week because the surface soil was too dry to favor expansion or sporulation. During periods in April and May when the soil was moist, soil temperatures were too high for the development of Botrytis spores from the neck rot lesions. Botrytis was active only when both humidity and temperature were favorable.

This was finally indicated by the reactivation of neck rot lesions and an epiphytotic of leaf spotting during the middle of May. The epiphytotic was introduced by 2 cloudy days and 1 foggy day, followed by 3 days of rain. Twenty-four hours after the rain began, neck rot lesions already carried an enormous load of spores. For 3 days both soil and air temperatures were within the optimum range for the rapid development of Botrytis, and moisture was ample. Dispersion of spores initiated hundreds of leaf spots on single leaves, but the leaf lesions were still very small when the rain stopped and temperatures rose. Further development of the disease was checked. Culturing from small leaf lesions some time later failed to reveal the presence of active Botrytis mycelium except in a very few instances, which illustrates once more the ephemeral existence of mycelium in the small leaf spots produced by *B. gladiolorum*.

(c) *Soil temperatures and neck rot in San Diego County*, 1950-51.—The main area for winter gladioli is at present in San Diego County. There during typical 4-week periods in the autumn, spring, and winter of 1950-51 continuous temperature records were taken in the surface 3 or 4 in. of the soil. The site chosen was a gladiolus planting a few hundred yards from the coast-line near Oceanside.

From November to March, temperatures in the surface soil ranged between 40°F. and 70°F. for weeks at a time. Neck rot developed on a fraction of 1 per cent of gladiolus plants about the middle of November, soon after mean surface soil temperatures first fell below 70°F. Lesions remained almost constantly active in the production of spores. During the next 4 months an increasing number of plants, in some lots up to 10 or 20 per cent, developed neck rot lesions, each capable of producing millions of spores.

As early as the middle of November daytime air temperatures as well as soil temperatures were near 60°F., and fogs or light rains were more or less continuous for nearly a week. An uncontrollable Botrytis epiphytotic developed on the leaves and flowers of this planting. Subsequently there was further spread of the disease whenever cool moist weather recurred. Towards the end of the winter season, when the weather became warmer and drier, Botrytis was successfully controlled in this area by roguing neck rot plants and spraying regularly every 5 days with zineb.

Eight or 9 mi. inland there was less continuous mist and rain, and in gladiolus plantings that were regularly sprayed with zineb there was relatively little Botrytis leaf spot or flower blight. Soil temperatures were much the same as in the coastal area. Except towards the end of winter the incidence of neck rot was almost as high as on the coast, even in plantings where Botrytis leaf spot was held in check by an adequate spray schedule.

The seasonal development of neck rot in these areas was similar during the winter of 1951-52.

Defensive reactions of host tissues.—The neck of the gladiolus plant consists of sheathing leaf bases, lacking chlorophyll, but it reacts to Botrytis infection in much the same way as the green leaf blade. Both aggressive and non-aggressive lesions may be formed, but aggressive lesions are normally in higher proportion than on the leaf blade. The longer continuation of humid conditions at and below ground level is sufficient to account for this difference.

The non-aggressive lesions are themselves evidence that leaf tissue is capable of a defensive reaction against invasion by Botrytis. Contents of affected cells precipitate in a gummy mass, and cell walls become brown. Gum formation is most evident in infected tissues submitted to warm temperatures and low humidities. Small superficial Botrytis lesions, after periods of exposure to warm dry weather, frequently contain subcuticular hyphae empty of contents and obviously dead. They are surrounded by dead epidermal cells full of wound gum. In several instances microscopic examination and cultures from single small lesions were made with material from the same infected leaves, and there was correlation between the visible condition of the hyphae and the number of lesions yielding *B. gladiolorum* in culture.

The intensification of wound-gum formation in invaded leaf tissue by warm conditions apparently reduces the maximum temperature at which Botrytis mycelium can actively parasitize gladiolus leaves. The work of McClellan et al (5), and that cited above, indicate 70°F. as the maximum level for the development of leaf lesions, yet *B. gladiolorum* grows rapidly in culture at 70°F. On the gladiolus plant itself, petal tissue responds to Botrytis infection by water-soaking, not by gum formation, and it is readily invaded at 70°F. In the field at warm temperatures, marginal for Botrytis disease, the most serious damage is often caused by invasion and rotting of flowers.

On gladiolus corms, temperatures as low as 55°F. can prevent development or extension of Botrytis rot (5). In the corms Botrytis hyphae penetrate most commonly to a subcuticular position or along vascular bundles (2). Beyond the invading mycelium, the walls of 4 or 5 layers of cells may be discolored. In nearby healthy cells a wound periderm layer rapidly forms, provided the temperature of the invaded corm is around 55°F. or higher (1). Botrytis mycelium cannot penetrate wound periderm. This additional reaction to invasion by Botrytis protects the corm from invasion at temperatures far below the optimum for growth of *B. gladiolorum* mycelium on artificial media (5). Protection is given in spite of the continued presence of living mycelium. Some infected corms in which Botrytis rot was entirely checked by curing at 95°F. readily yielded *B. gladiolorum* in cultures from the original lesions.

SUMMARY

Neck rot lesions produced by *Botrytis gladiolorum* Timmermans develop mainly from inoculum carried on corms or from air-borne spores.

Typical neck rot consists of "aggressive" and persistent lesions, similar to aggressive lesions on the green leaf blade.

Numbers of spores of the order of 1 million may be produced from single neck rot lesions during 24 or 48 hours of favorable weather.

A field study of neck rot in a crop relatively free from the leaf spot phase of Botrytis indicated that continued high humidity and temperatures around 55°–65°F. favor the development of neck rot. These are the same conditions that favor the leaf spot phase of Botrytis disease, but they exist at soil level and below rather than above. Subsequent observations confirmed these findings.

Histological studies suggested the protective mechanisms, by which gladiolus tissues limit invasion by Botrytis mycelium, include gum formation in the leaves and periderm formation in the corms. The effectiveness of these protective reactions seems to be related to temperature.

No indication was seen of a protective reaction in petal tissue.

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University of California, Los Angeles

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DEVELOPMENT OF THE ROOT-KNOT NEMATODE, MELOIDOGYNE INCOGNITA, AS AFFECTED BY POTASSIUM NUTRITION OF THE HOST[1]

Bakir A. Oteifa

In a previous paper it was shown (4) that the root-knot nematode, *Meloidogyne incognita*, (Kofoid and White, 1919) Chitwood, 1949 had a marked effect on the mineral nutrition of the host plant, and that the damage resulting from infection by these pests could be reduced by increasing the level of potassium supplied to the host. In the present paper the influence of host nutrition, in particular the potassium levels, on the comparative development of this parasite will be discussed.

Development of root-knot nematodes is influenced by temperature as well as by the type of host plant. Tyler (6) made an extensive study of the effect of temperature on nematode development. She found that in tomato plants, grown in soil at 27°C., 16–19 days elapsed from the time larvae entered the roots until females began to lay eggs. When the plants were grown in soil at 24°C., this interval was 25–27 days, and at 15.5°C. it was 67 days. Demonstrating the effect of the host on parasite development, Godfrey and Oliveira (2) found that in cowpea plants grown at a temperature permitting rapid development, the interval between penetration of larvae and first deposition of eggs was 19 days, but in pineapple, grown under identical conditions, it was 35 days.

Little attention, however, has been given to the influence which host nutrition may have on the development of such parasites. Tyler (5) investigated the relation of nutrition to the development of the root-knot nematode in root cultures. She postulated that the number of eggs laid, and the number, size, and vigor of larvae are affected by the nutritional status of the host and concluded that a healthy condition of the host is an important factor for the development of its parasite.

[1] Accepted for publication December 10, 1952.

Division of Plant Pathology, University of Maryland and Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, on leave from Fouad 1. University, Egypt.

Materials and methods.—Nematode inoculum, of the species *Meloidogyne incognita*, was maintained on the Rutgers variety of tomato, *Lycopersicon esculentum* Mill, on which production of egg masses readily occurs. Egg masses were carefully selected and several representatives were checked under a dissecting microscope to insure the presence of the infective second stage larvae.

Seeds of lima bean plants, *Phaseolus lunatus* L. var. Henderson were germinated in pure quartz sand. When in the 3 or 4 leaf stage each plant was transplanted to a small pot containing 10 egg masses. After 3 days the seedlings were removed and the roots were washed free of sand and rinsed in tap water. Such seedlings were then retransplanted to glazed 1-gal. crocks filled with sterilized pure quartz sand. Nutrient treatments were initiated immediately after transplanting. The effect of 3 levels of potassium representing low, optimum, and excessive concentrations on the development of the parasite was studied. Composition and preparation of the nutrient solutions applied were reported in a previous paper (4). Applications of 250 ml. were made every other day. Crocks were flushed weekly with tap water to prevent any accumulation of salts. Air temperature in the greenhouse was 65–85°F with an average of 75°F. All plants were subjected to the same temperature ranges during the periods of observation.

Every other day, 1 plant was removed from the crock, the sand washed free from the roots, and the entire root system was fixed with a modification of Flemming's formula.[2] In preparation of the solution, 6 cc. of distilled water, 10 cc. of 1 per cent chromic acid, 10 cc. of 10 per cent acetic acid, and 2 cc. of 2 per cent osmic acid were combined and heated to ap-

[2] The technique employed was suggested by H. W. Reynolds, Division of Nematology, U. S. Cotton Field Station, Sacaton, Arizona.

TABLE 1.—*Development time of the root-knot nematode, Meloidogyne incognita, in lima bean plants supplied with deficient, optimum and excessive potassium nutrient levels*

Number of days after inoculation	Number of parasites in each group														
	Deficient K					Optimum K					Excessive K				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
4	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0
6	97	3	0	0	0	93	7	0	0	0	87	13	0	0	0
8	83	17	0	0	0	82	18	0	0	0	77	23	0	0	0
10	77	23	0	0	0	35	45	0	0	0	35	52	13	0	0
12	66	20	4	0	0	17	75	8	0	0	7	68	15	10	0
14	41	47	12	0	0	15	63	22	0	0	3	41	40	16	0
16	29	47	24	0	0	11	31	52	6	0	0	22	24	23	31
18	16	42	38	4	0	8	21	64	7	0	0	0	39	17	44
20	16	33	52	9	0	5	16	66	13	0	0	0	3	27	70
22	12	19	55	14	0	0	12	69	19	0	0	0	0	6	94
24	9	21	50	19	0	0	5	47	28	20					
26	4	13	45	26	0	0	0	17	35	48					
28	1	19	54	27	0	0	0	3	13	84					
30	0	9	48	33	0	0	0	0	8	92					
32	0	5	65	30	0	0	0	0	2	98					
34	0	2	54	42	0										
36	0	0	49	61	0										
38	0	0	32	68	0										
40	0	0	28	70	2										
42	0	0	28	67	5										
44	0	0	19	70	11										
46	0	0	11	54	35										
48	0	0	9	55	46										
50	0	0	5	44	51										
52	0	0	4	37	59										
54	0	0	0	12	88										

proximately 55°C. Roots were immersed for about ½ hour, then washed in running water for at least 4 hours. They were then transferred to 15 per cent ethyl alcohol for 15 minutes, 30 per cent for ½ hour, 50 per cent for 1 hour and absolute alcohol for 2 hours. The dehydrated roots were then cleared in methyl salicylate. This technique was satisfactory for staining the parasites and clearing the root tissues. A small portion of the root system was clipped off and examined under a dissecting microscope. Every parasite contained in this portion of the root system was classified and recorded according to Christie's groups of stages of development (1), (Fig. 1, A to E). Examination of roots continued until 100 parasites had been counted from each plant.

RESULTS OF EXPERIMENTS.—Results are in Table 1. The parasite development was slower in lima bean plants receiving a deficient potassium nutrition level than in plants with either optimum or excessive potassium levels. While the period that elapsed from the time of inoculation until the parasites developed through stages A, B, and C was approximately identical in the 3 nutrient treatments, the period required for further development was considerably influenced by the nutrition of the host plant. The nematodes reached the fully grown stage D (Fig. 1, D) after 18, 16 and 12 days after inoculation under deficient, optimum and excessive potassium respectively. The period required for egg deposition was also considerably affected by variations in the levels of potassium supplied to host. In this respect the time that elapsed from stage D, in which the females are fully grown (Fig. 1, D), to stage E in which eggs are deposited (Fig. 1, E), was 22, 8, and 4 days under deficient, optimum and excessive levels of potassium respectively.

It is evident from the table that the number of parasites which reached each stage was greater in plants receiving excessive and optimum potassium treatment than the number in plants receiving the deficient potassium treatment. No differences, however, were noticed in the sizes of the parasites under these conditions.

DISCUSSION. — Root-knot nematodes, *Meloidogyne* spp., are obligate parasites, and the host plant is a requisite for the completion of their life cycle. Development of these parasites has been reported to be influenced mainly by 2 factors, temperature (6) and type of host plant (2). One of the most significant results from the present study has been the demonstration that the nutritional status of the host plant also plays an important role in influencing the length of life cycle of the root-knot nematodes. Table 2 is a condensed comparison of lengths of time required for developmental stages in the lima bean plants when supplied with 3 different levels of potassium concentrations.

It is evident from this table that the greatest difference among the 3 nutritional treatments lies in their influence on length of time required for the development from the fully grown female stage D to the egg-laying stage E. The first eggs were not observed until about 40 days from the time of inoculation in the deficient potassium series, 24 days in the optimum potas-

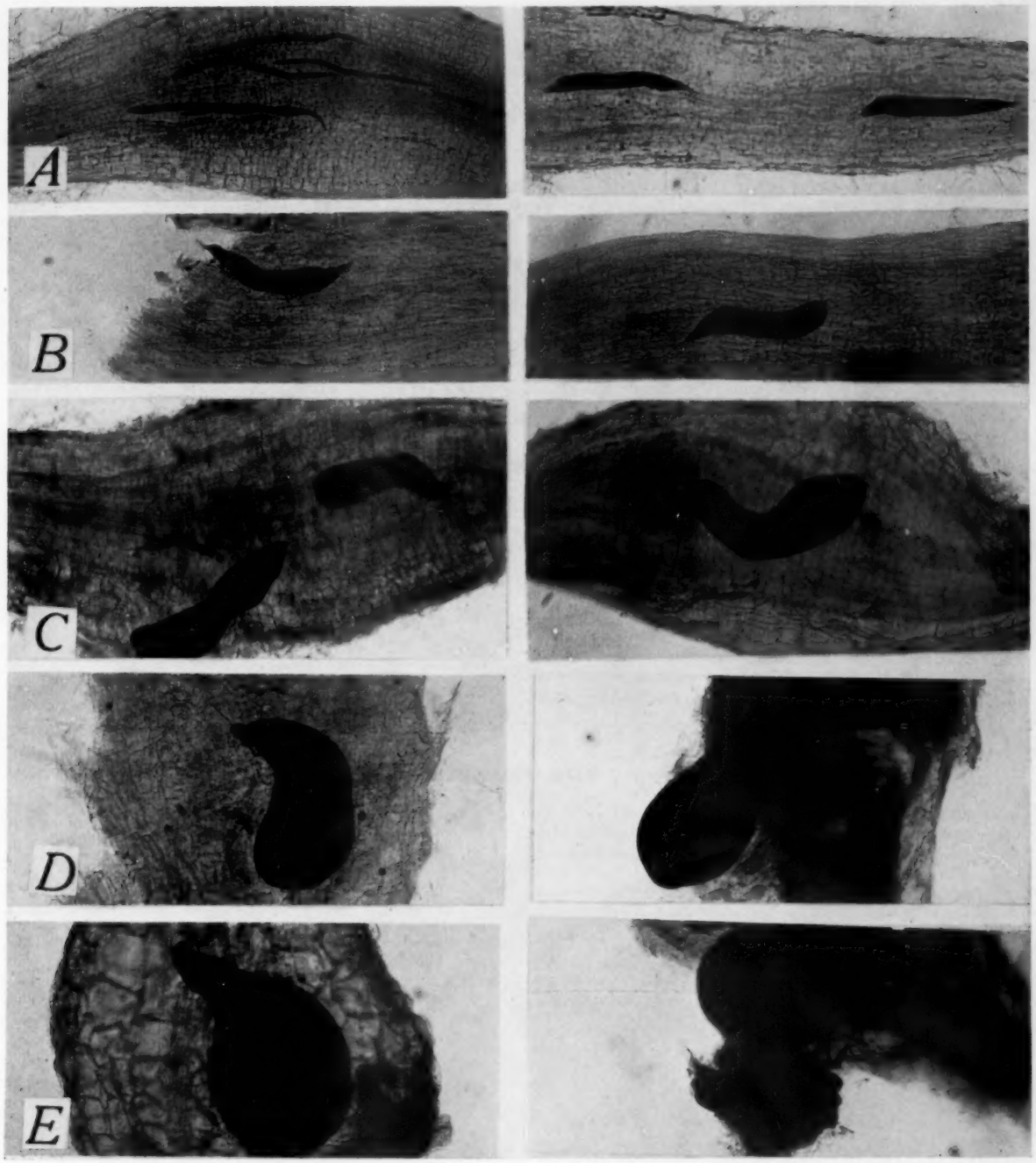

FIG. 1. Developmental stages of the root-knot nematodes, *Meloidogyne incognita*. A) includes larvae in the early stages of development; B) includes larvae which have acquired a more or less hemispherical posterior end, terminated by a spike, to larvae which are about to complete the final molt; C) includes females which have completed the molts to those which have not yet developed the typical adult shape; D) includes females of typical adult shape, but which have not yet deposited eggs; E) includes females which have begun to deposit eggs.

sium series, and 16 days in the excessive potassium series. Such data indicate that the nutritional condition, particularly potassium host nutrition, is of considerable importance in determining development time of the root-knot nematodes.

Previous data (3) showed that as the potassium supply was increased the rate of oviposition of this nematode was correspondingly increased. Present data indicate that at least a portion of the increase in egg production is due to the shortened life cycle. Thus, the application of potassium will promote the build up and maintenance of nematode populations. On the

TABLE 2.—*Comparative development times of the root-knot nematode, Meloidogyne incognita, as influenced by variations in the amount of potassium supplied to the host plant*

Stages of development	Period of development in days		
	Deficient K	Optimum K	Excessive K
From stage A to stage B	6	6	6
From stage B to stage C	6	6	4
From stage C to stage D	6	4	2
From stage D to stage E	22	8	4
From inoculation;			
To first egg production	40	24	16
To 50% of females depositing eggs	50	26	20
To 85% of females depositing eggs	54	30	22

other hand, as previously shown by experimental data (4), damage caused by root-knot nematodes can be reduced by increasing the amount of potassium available to the host plant.

Thus excessive application of potassium favors the rapid development and reproduction of the nematode, and at the same time protects the plants to some extent from nematode injury. A possible explanation is that a considerable amount of potassium is required for the nutrition and egg production of this nematode, as well as being an important mineral element for the growth of the plant. Hence, potassium fertilizers applied to plants growing in nematode infested soils would increase plant growth, provided the soil is deficient in this element. Unfortunately, applications of potassium also serve to promote increase of the root-knot nematode population.

SUMMARY

In experiments demonstrating the effect of plant host nutrition on the development of a root-knot nematode *Meloidogyne incognita*, 3 potassium nutrient levels representing deficient, optimum, and excessive concentrations were employed. Only minor differences in the development of the nematodes in the 3 treatments were noted between entry of the larvae into roots and development to fully grown females. Potassium nutrition had a considerable effect, however, on the length of time between development of the female to maturity and egg deposition. First egg production was observed after 40, 24, and 16 days from the time of inoculation, in the deficient, optimum, and excessive potassium series, respectively. The findings indicate that potassium nutrition, is of considerable importance in determining the development time of root-knot nematodes.

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STUDIES ON STONE-FRUIT VIRUSES IN CUCURBIT HOSTS. I. A METHOD OF EVALUATING THE INFECTIVITY OF INFECTIOUS JUICE [1]

R. S. Willison and M. Weintraub

Until recently, the lack of herbaceous hosts for stone-fruit viruses, the practical difficulties in rearing quantities of woody plants, and the virtual impossibility of juice transfers of virus to Prunus hosts have seriously limited studies of the nature and properties of the viruses themselves. These limitations have in some measure been removed by the discovery[2, 3] that virus entities in sour cherry trees affected with either cherry yellows or necrotic ringspot can be mechanically transmitted to cucumber and other cucurbit hosts. Accordingly, an investigation of the behavior and properties of those stone-fruit virus entities that are transmissible to cucumber has been undertaken.

One of the first requirements for such a study is a quantitative method of evaluating the infective capacity of, or relative concentration of virus in, a given inoculum. The method of counting local lesions has not been practicable in these studies as no suitable local lesion host for stone-fruit viruses is yet available. Local lesions are usually formed on rubbed cucumber cotyledons, but, even with the use of abrasive dusts, they are rarely numerous enough to be useful for quantitative determinations. Moreover, these primary lesions are sometimes discrete and sometimes diffuse, and may even take the form of veinclearing or veinbanding. As a substitute for local lesion counts a system of infection rating, based on severity of symptom expression and the ratio of infected to inoculated plants, has been devised.

The degree of severity of the disease induced by stone-fruit viruses in cucurbit hosts depends on the isolate used but also varies markedly with the conditions under which the inoculated plants are grown. In cucumber plants inoculated on 1 cotyledon during the development of the first true leaf, differences in response to infection by individual isolates are readily recognizable in 10–14 days. At that time reactions range from well-defined systemic symptoms and relatively little stunting, through more or less complete suppression of growth to death of the apical growing point and some or all of the top of the plant. When the concentration of virus in a given inoculum is high enough to provide dosages well above the minimum required for infection, the characteristic reaction can be expected on all the plants inoculated. However, with lower virus concentrations some of the inoculated plants escape infection, whereas in others the disease runs its normal course. Furthermore, in plants receiving dosages close to the effective minimal level, the course of infection may stop at some intermediate point. Cucumber plants tend to become less susceptible as they grow older. Consequently, the quantity of virus introduced into points of entry may be so small that, although infection is established, the virus does not multiply rapidly enough for complete systemic distribution while the plant is in a more susceptible stage.

These considerations suggest that the over-all severity of the disease induced in a group of plants can be used as a measure of the degree of infectivity of a given inoculum. The degree of infectivity in turn reflects the relative level of effective virus concentration in the inoculum. The infection rating, as defined above, is obtained by assigning values to the various phases of symptom expression and averaging the resulting scores for individual assay plants inoculated in replicate with a given inoculum (Table 1). The infection rating has also proved useful for comparing different isolates and the effects of various treatments on individual preparations as well as the effects of environmental conditions and other factors on the course of infection in assay plants. Examples of these applications are presented below.

TABLE 1.—*Scoring values used in determining infection ratings for in vitro preparations of stone-fruit virus isolates*[a]

Symptom	Score A	Score B[b]
Primary lesions on rubbed cotyledons	1	
Systemic symptoms mild	–	1
Systemic symptoms well-defined	2	
Growth suppressed	–	1
Growing points, etc. killed	2	

[a] Infection rating $= \Sigma s/n$, where s = scores for individual plants and n = number of plants inoculated.

[b] Scores used for intermediate reactions, not applicable when more severe symptoms occur.

In practice, infection ratings are read at periodic intervals after the inoculation of assay plants. However, assessments and comparisons are usually made with end-points or stabilized ratings as indicated by the absence of significant changes in successive ratings. Ratings usually become stable 10–14 days after inoculation. Scoring can be done quickly and accurately, except occasionally in defining the limits between mild and well-defined symptoms. Doubts on this point have almost invariably been resolved at the subsequent reading.

The isolates used in the examples given below were G.1 and Y.1 obtained from 2 cherry-trees showing

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Contribution No. 1175 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Boyle, J. S., J. D. Moore, and G. W. Keitt. 1949. Comparative studies of a virus disease of cucumber transmitted mechanically from sour cherry and several cucumber virus diseases. (Abs.) Phytopathology 39: 2.

[3] Moore, J. D., J. S. Boyle, and G. W. Keitt. 1948. Mechanical transmission of a virus disease to cucumber from sour cherry. Science 108: 623-624.

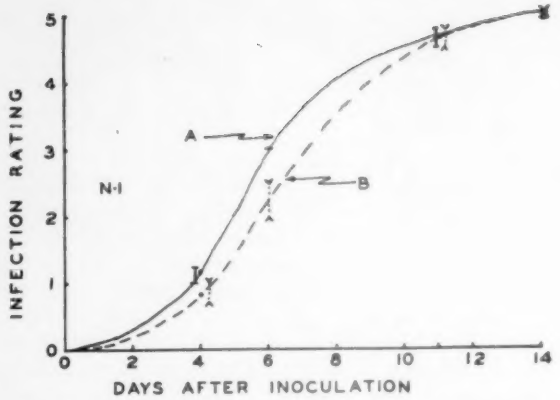

FIG. 1. Progressive changes in infection ratings on assay plants inoculated with isolate N.1. A) ranges of ratings obtained with 3 aliquots of inoculum from cucumber plants infected for 11 days before grinding. B) as in A, but inoculum from plants infected for 25 days before grinding. Assay plants inoculated at the same time and grown under the same conditions.

symptoms of green ring mottle and cherry yellows respectively, N.1 from Lombard plum with latent necrotic ringspot, and T.2 from sweet cherry with tatter leaf.

MEASURING THE PROGRESS OF INFECTION.—Two lots of inoculum of isolate N.1 were prepared from cucumber sources A and B infected for 11 and 25 days respectively. Each inoculum was divided into 3 aliquots, diluted to ¼. Six plants were inoculated with each aliquot immediately after dilution. All assay plants, grown under the same conditions before and after inoculation, became infected. Infection ratings were read on the 4th, 6th, 11th and 14th days. The range of ratings obtained at each reading with the 3 aliquots of each inoculum is indicated in Fig. 1 by the length of the vertical lines across the curves. It is evident that the agreement between the aliquots of inoculum A was extremely close throughout. Difference between the aliquots of inoculum B were somewhat wider but were not significant (maximum difference 0.5 ± 0.3, $P = .25$). The divergence between A and B on the 6th day was significant (difference 0.8 ± .12, $P < .01$)

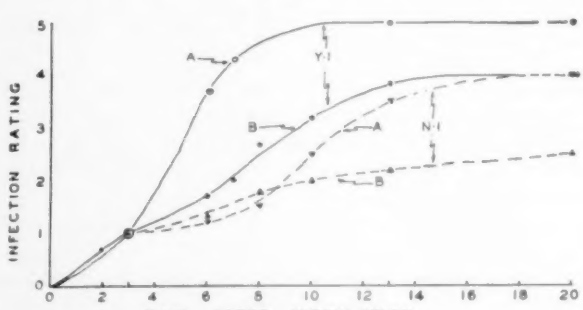

FIG. 2. Progressive changes in infection ratings on assay plants inoculated with isolates N.1 and Y.1 and grown at 75°F. but A and B illuminated daily for 10 and 18 hours respectively. Inoculum from cucumber plants infected for 14 days before grinding.

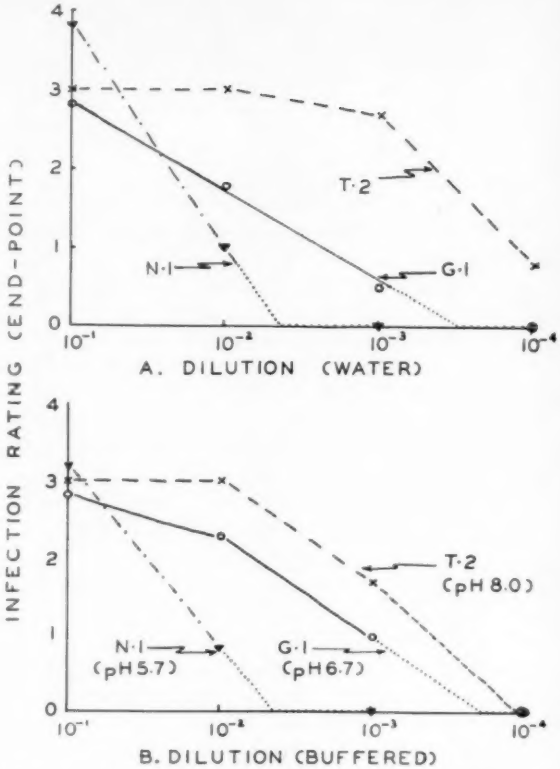

FIG. 3. Representative dilution curves for isolates, G.1, N.1 and T.2. A) diluted with distilled water, B) with phosphate-citric-acid buffer.

and indicated that the effective concentration of virus was measurably lower in the inoculum from the older source. This view was confirmed by the differences observed between the end-points for the diseases induced by inocula A and B in a test made after an aging period of 4 hours at 0°C.

EFFECT OF ENVIRONMENT ON THE INDUCED DISEASE.—Two sets of 12 plants each were inoculated at the same time with isolates N.1 and Y.1 respectively. Both inocula were prepared from cucumber sources infected for 14 days. Six plants of each set were placed in room A maintained at 24°C. and illuminated 10 hours daily, and 6 in room B at the same temperature but illuminated 18 hours daily. All plants became infected. The progress of infection in each set of plants, measured at intervals as above, showed that significant differences developed between plants inoculated with the same inoculum, but assayed under different conditions (Fig. 2). Similarly, differences also occurred between plants inoculated with different isolates, but assayed under the same conditions. Furthermore, characteristic reactions to the 2 isolates could be compared equally well under either set of conditions.

EFFECT OF DILUTION.—During grinding operations distilled water was added at the rate of 1 ml. per gram of leaf. 2-ml. aliquots of the mixture were then brought up to 10 ml.—approximately 10^{-1} dilution—

by the addition of either distilled water or 1 per cent dipotassium phosphate buffer adjusted to the required pH levels with citric acid. Further dilutions were made in the usual way. Representative dilution curves for isolates G.1, N.1, and T.2 were derived from stabilized infection ratings (Fig. 3A and B). Numbers of inoculated and infected assay plants are given in Table 2, along with the maximum possible rating for each isolate. The pH levels represented in Fig. 3B were those which gave the highest over-all ratings for the respective isolates in the complete experiment of which these results were part. Data are comparable for individual isolates, but not for different isolates which were assayed under different conditions. It should be noted that progressive changes in effective virus concentration were reflected by relatively uniform changes in infection rating. Also the 3 isolates differed in their response to the changes in the reaction of the inoculum.

TABLE 2.—*Proportions of assay plants infected by isolates G.1, N.1 and T.2 at different dilutions and possible maximum infection ratings*

	Plant infected [a]								
	Water dilutions				Buffer dilutions				Max.
Isolate	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Rating[b]
G.1	5/5	4/6	1/6	0/6	6/6	5/6	3/6	0/6	3
N.1	4/4	2/4	0/4	0/4	5/5	1/5	0/5	0/5	4
T.2	6/6	6/6	6/6	2/6	6/6	6/6	4/6	0/6	3

[a] Numerator, no. plants infected; denominator, no. plants inoculated.

[b] Maximum infection rating possible for the given isolate under the conditions of assay.

DISCUSSION.—For any valid method of quantitative assay, results should be reproducible. In the field of study under consideration, however, reproducibility within narrow limits is difficult both to attain and to demonstrate, because of the variability of reaction with changes in environment and other factors. However, 2 examples have been given in which no significant differences occurred in assay by the infection rating method when similar sets of plants were inoculated in triplicate with the same inoculum and grown under the same conditions. On the other hand, it has also been shown that significant differences, some of which are relatively slight, can be obtained: 1) with the same isolate assayed under the same conditions when using inoculum from similar sources inoculated for different lengths of time; 2) when different isolates were assayed under the same conditions; and 3) when the same inoculum was assayed under conditions in which 1 factor varied. The dilution curves presented above also indicate that changes in virus concentration in an inoculum can be measured with a reasonable degree of accuracy. The method of assay by infection rating is therefore considered to be sensitive and accurate enough for the purpose for which it was designed. At the present time, it is the only quantitative method which can be applied to the study of stone-fruit viruses.

Like other quantitative methods, the infection rating has its limitations. In the first place, its working range is relatively narrow, being restricted to levels of effective virus concentration below that giving maximum rating. Consequently, the effects of different factors on the infectivity of a given inoculum cannot always be followed when the virus concentration in the inoculum is well above the critical level. Another apparent disadvantage is that the maximum rating typical of 1 isolate may not be the same as that of another isolate under the same conditions. Likewise, the maximum rating for any given isolate may vary considerably with conditions of assay. The variability of maximum rating is useful either for comparing different isolates or for comparing the effects of environment on reaction to a single isolate, but not for comparing relative virus concentrations. For example, under a given set of conditions, isolates A and B may give maximum ratings of 3 and 5 respectively, yet may be at the same level of concentration in the inocula. Such difficulties, which are also encountered in the use of other *in vivo* methods of assay, can be overcome by the proper design of experiment, in the one case, by adjustment of dilution of inoculum and, in the other, by determining dilution-end points.

Experience with the method has indicated that results are not appreciably affected by minor variations in inoculating technique, such as the quantity of abrasive dust used and the number of strokes made in applying inoculum to the cotyledon. Differences may occur in the susceptibility of individual cucumber seedlings, but their effects are minimized by the use of 1 variety, in these experiments Windermoor Wonder, and by replication.

SUMMARY

A quantitative method of assay by infection rating is described for use in investigating the properties of the stone-fruit virus entities affecting cucurbit hosts. The infection rating is the average degree of severity of the disease induced in a number of plants of uniform age inoculated with the same inoculum at the same time. Examples are given of the application of the method to the comparison of isolates, to the effect of conditions of assay on the reaction of plants to infection with the same isolate or with different isolates, and to the determination of changes in levels of effective concentrations of virus with dilution.

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PHYSIOLOGIC SPECIALIZATION IN PUCCINIA MENTHAE PERS., AND NOTES ON EPIPHYTOLOGY[1]

John W. Baxter[2] and George B. Cummins

The mint rust fungus, *Puccinia menthae* Pers., is an autoecious, full-cycle rust which occurs on wild and cultivated members of the family Labiatae throughout the world. Under conditions favorable for development of the disease, mint rust frequently causes serious damage in fields of commercially grown mint. In 1950 the disease was serious in Indiana and Michigan on spearmint, *Mentha spicata* L., and Scotch mint, *Mentha cardiaca* Baker. In 1949 and 1950 it attained epiphytotic proportions in Oregon and Washington on peppermint, *Mentha piperita* L. The absence of rust on peppermint in Indiana and Michigan has suggested the possible existence of physiologic races in this area.

P. & H. Sydow (5) listed 49 species in 16 genera of the Labiatae as hosts of *Puccinia menthae* and suggested that this rust might prove to be a "Sammelspecies." They noted certain differences in teliospore morphology among the forms occurring on different hosts, an observation which was confirmed by Fischer (3). Cruchet (2) conducted cross-inoculation experiments with European collections of *P. menthae* and differentiated 8 distinct physiologic races. Niederhauser (4) reported 6 physiologic races of the mint rust fungus in the state of New York.

The present paper reports the results of an investigation of physiologic specialization within *Puccinia menthae* in North America. It also presents field observations on epiphytology of mint rust in the mint-growing areas of northern Indiana.

MATERIALS AND METHODS.—In the present investigation 2 series of inoculations were made, using a total of 31 different hosts and 22 collections of *P. menthae*. Hosts and rust collections employed in the tests are listed in Table 1.

The differentiation of races is based on the following types of host reaction:

0 *Immune.* Flecking or small necrotic spots; no uredia formed.

1 *Highly resistant.* Uredia few, small, always in necrotic spots, some uredia failing to rupture the epidermis.

2 *Moderately resistant.* Uredia fairly abundant, of moderate size, occasionally a few small, in necrotic or occasionally chlorotic spots, frequently soon followed by telia.

3 *Moderately susceptible.* Uredia abundant, of moderate size. No necrosis produced, but sometimes slight chlorosis immediately surrounding the uredia.

4 *Highly susceptible.* Uredia abundant, large. No necrosis or chlorosis.

Collections used in these tests were purified by the single-pustule method. Resulting cultures were kept isolated from one another in large muslin-covered glass cylinders or under lantern globes. As soon as an adequate supply of inoculum had been built up, healthy, rust-free plants were inoculated in sets of 12, each plant being a different host. Each set contained 1 plant representing the host from which the inoculum had been obtained. Twelve additional plants were used as noninoculated checks. Inoculations were made by applying urediospores to the lower surface of the leaves with a scalpel. Two opposite leaves on each plant were inoculated, after which the plant was sprayed with water. The plants were kept in a moist chamber for 18 hours, then transferred to a greenhouse maintained at 18°–25°C. The types of infection were recorded 11 or 12 days after inoculation and again 3 or 4 days later. There were 3 replications in time of each inoculation.

RESULTS.—Results of the inoculations are in Table 1. The upper part of Table 1 shows the reactions obtained by testing each race only on the hosts which served as sources of inoculum. Of the 22 collections tested, 15 proved to be distinct from one another on the basis of reactions produced on certain hosts. Race 2, originating on *Mentha piperita*, produced a susceptible reaction on 12 different hosts (Table 1). Extremely narrow specialization was shown by races 4, 5, 6, 7, and 9. Results in Table 1 show further that *Mentha arvensis* and 3 species of *Satureja* exhibited wide susceptibility, while 1 strain of spearmint, designated P-13[3], proved to be immune from all races employed in the tests.

FIELD OBSERVATIONS.—Observations in the mint-growing areas of Indiana during the spring and summer of 1951 revealed that the aecia of *P. menthae* may appear on cultivated spearmint as early as April 15 and on escaped spearmint as early as May 5. The first aecia appeared on mint which had been left unplowed throughout the winter. These aecia occurred chiefly on the above-ground portions of the shoots, while in fall-plowed fields most of the aecial lesions were found on the below-ground portions, at depths ranging from ½–6½ in. below the surface of the soil.

Underground aecial lesions were found as late as May 29 in most of the fields under observation, while newly formed above-ground aecia were observed as

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[3] This strain was selected by Dr. R. C. Baines, formerly of the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

TABLE 1.—*Infection types produced by 15 races of Puccinia menthae.*[a]

Hosts inoculated	Races of P. menthae														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Mentha cardiaca* L. (Scotch)	4	2	0	0	0	0	0	0	0	0	3	4	0	0	1
2. *Mentha piperita* L.	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1
3. *Mentha arvensis* L.	4	4	4	0	0	0	0	0	0	0	4	4	0	0	4
4. *Monarda fistulosa* L.	0	0	0	4	0	0	0	0	0	1	0	0	0	0	0
5. *Monarda punctata* L.	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
6. *Monarda didyma* L.	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
7. *Blephilia hirsuta* Benth.	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
8. *Monardella villosa* Greene	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
9. *Satureja vulgaris* Fritsch	3	4	3	–	–	–	–	–	4	0	3	3	3	–	3
10. *Cunila origanoides* Britt.	0	3	0	–	–	–	–	–	0	4	0	0	0	0	0
11. *Mentha spicata* L.	3	0	0	0	0	0	0	0	0	0	4	0	0	0	0
12. *Mentha cardiaca* L.	4	2	0	0	0	0	0	0	0	0	3	4	0	0	1
13. *Pycnanthemum pilosum* Nutt.	0	0	0	–	–	–	–	–	0	0	0	0	4	0	0
14. *Pycnanthemum virginianum* L.	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
15. *Mentha* sp.	0	4	3	–	–	–	–	–	0	0	0	2	0	0	4
Mentha spicata (P-13)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mentha arvensis var. *piperascens* Mal.	4	4	4	0	0	0	0	0	0	0	4	4	0	0	4
Mentha crispa L. (Strain A)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mentha crispa L. (Strain B)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Mentha crispa L. (Strain C)	0	4	0	–	–	–	–	–	0	0	0	0	0	0	–
Mentha citrata Ehrh.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mentha rotundifolia Huds.	2	0	0	0	0	0	0	0	0	0	3	0	0	0	0
Mentha longifolia Huds.	0	4	0	0	0	0	0	0	0	0	0	0	0	0	3
Mentha aquatica L.	0	3	0	–	–	–	–	–	0	0	0	0	0	0	0
Mentha pulegium L.	0	0	–	–	–	–	–	–	–	–	0	–	0	–	–
Monardella sp.	0	1	0	1	0	0	0	4	0	0	0	0	0	0	0
Hedeoma drummondii Benth.	3	0	0	0	0	0	0	0	0	0	–	0	–	–	–
Hedeoma hispida Pursh.	0	3	0	–	–	–	–	–	0	0	–	–	3	–	0
Satureja douglasii Brig.	3	4	3	–	–	–	–	–	3	0	4	3	3	3	3
Satureja hortensis L.	3	4	3	–	–	–	–	–	3	0	4	3	3	3	3
Satureja calamintha Scheele	0	0	0	–	–	–	–	–	0	0	0	0	0	0	0
Thymus vulgaris L.	0	0	0	–	–	–	–	–	0	0	0	0	0	0	0

[a] Dash indicates that no inoculation was made; 0, immune; 1, highly resistant; 2, moderately resistant; 3, moderately susceptible; 4, highly susceptible.

late as June 15. The latter were on systemically infected shoots which had arisen from nodes ½–2 in. below ground level. These above-ground aecia did not rupture the epidermis and liberate aeciospores until the first week in July. The aeciospores appeared to be relatively ineffective in long-range spread of the disease, since no first-generation uredia were found at distances greater than 18 in. from the source of aeciospores.

The first uredia observed in 1951 under field conditions were found on June 15. The age of these uredia indicated that infection must have occurred about June 1. On June 20, heavy uredial infection was observed in 2 fields from which transplants had been pulled during the first week in June. Spore traps, set up around 1 of these fields and checked at weekly intervals throughout the summer, provided evidence that urediospores of *P. menthae* may be carried at least 2 mi. in the general direction of the prevailing winds.

DISCUSSION.—The results of the experiments reported in this paper agree in some respects with those obtained by Cruchet (2) in his work with European collections of mint rust. At several points, however, distinct differences are evident. These may be due in part to misidentification of host plants, at least within the genus *Mentha*, in which extreme variability and the existence of numerous hybrids often make exact identification difficult or impossible. It is possible that the physiologic races reported in the present paper are all distinct from those differentiated by Cruchet.

Results in Table 1 show that the races commonly found on spearmint are not capable of infecting peppermint. This confirms the observations of mint growers in Indiana and Michigan who have frequently noticed that peppermint plants remained free of rust even when directly adjacent to heavily rusted spearmint.

The races reported here as occurring on *Mentha piperita* and *Mentha spicata* have proved to be less specialized than those collected by Niederhauser (4) on these hosts. In his inoculations infection was obtained only on plants of the same species or strain as those from which the inoculum was taken, which led Niederhauser to doubt the importance of wild species as sources of inoculum in epiphytotics of rust on cultivated mints. Results obtained by the writers do not support this view, since it has been found that *Mentha arvensis*, *Cunila origanoides*, and species of *Hedeoma* and *Satureja* are susceptible to 1 or more races which

occur on cultivated spearmint or peppermint. Not all of these species, however, can be considered important potential sources of inoculum. For example, *Cunila origanoides, Hedeoma hispida,* and *Hedeoma drummondii* are all restricted as to habitat and geographical distribution and do not commonly occur in areas of commercial mint production. *Satureja hortensis* and *Satureja vulgaris*, although susceptible to most if not all races on cultivated mints, are not important sources of inoculum. These species, introduced from Europe, are sparingly distributed in North America and not abundant in the vicinity of commercial mint fields. *Satureja douglasii*, on the other hand, may be important as a potential host for the race on peppermint in the Pacific Coast mint-growing region.

Table 1 shows that *Mentha arvensis* proved highly susceptible to the races collected on the cultivated mint species. Of the wild hosts this species is probably of the greatest potential importance as a host for the races which attack cultivated spearmint or peppermint. It is widely distributed throughout the northern hemisphere along streams, in wet meadows and swamps, and along ditch banks, and probably should not be overlooked as a possible source of inoculum when growing near fields of cultivated spearmint or peppermint.

The P-13 strain of spearmint shows promise as a possible source of rust immunity (Table 1). Since it is male fertile, this mint might form the basis for the development of a desirable, rust-immune spearmint strain by breeding and selection.

The 15 physiologic races reported here probably represent only a portion of the total in North America. It is possible also that in some or all the tests in which 2 or more collections were found to represent a single race, the use of additional differential hosts would have revealed previously undetected differences. The writers wish to emphasize the fact that most of the differentials used in these tests were at the species level, so that the results are not directly comparable to those obtained, for example, in testing collections of leaf rust on differential varieties of wheat. It seems probable that several if not all races reported herein are actually "super" races capable of being subdivided by the use of appropriate differential hosts.

Field observations indicate that the primary inoculum for epiphytotics of mint rust in northern Indiana consists of aeciospores, which may be produced at least as early as April 20 and as late as the first week in July. While it is probable that most epiphytotics of mint rust in this area are initiated by aeciospores from above-ground lesions, it seems possible that some heavy rust infections can be traced to aeciospores dislodged from aecia on the rhizomes during transplanting.

While the writers have not obtained experimental data on which to base recommendations for control of mint rust, certain suggestions regarding control can be made on the basis of the field observations. These observations point to deep fall plowing, with thorough burial of plant debris, as a necessary measure in controlling the disease. This practice would probably be of value not only as a control measure for mint rust, but also for mint anthracnose, as suggested by Baines (1). Other measures which appear advisable are the elimination of exposed debris along back-furrows and dead-furrows and the destruction of nearby escaped or wild mints which can serve as hosts for the races of *P. menthae* infecting commercially grown mint.

SUMMARY

A study of physiologic specialization in the mint rust fungus, *Puccinia menthae* Pers., showed that this species is composed of at least 15 physiologic races in North America.

Susceptibility to one or more races infecting cultivated mints was shown by *Satureja vulgaris, Satureja hortensis, Satureja douglasii, Hedeoma hispida, Hedeoma drummondii, Cunila origanoides, Mentha arvensis* and an unidentified wild species of *Mentha.*

The P-13 strain of spearmint proved to be immune from all 15 races.

Field observations in northern Indiana during 1951 revealed that aecia of *P. menthae* may appear on cultivated spearmint as early as April 20 and as late as June 15, and on escaped spearmint as early as May 5.

Underground aecial lesions were found on stems and rhizomes at depths ranging from ½–6½ in. below the surface of the soil.

Deep fall plowing, elimination of exposed plant debris, and destruction of nearby wild or escaped hosts are suggested as possible measures in the control of mint rust.

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A PROPOSED SPECIES CONCEPT IN THE SMUT FUNGI, WITH APPLICATION TO NORTH AMERICAN SPECIES [1]

George W. Fischer and Charles Gardner Shaw

That the problem of defining and delimiting species is an old and general one is well attested by the vast amount of literature on the subject. The concept of a species has undergone various mutations and evolutionary changes since the comparatively uncomplicated era when Linnaeus first established and so thoroughly employed this all-important taxon.

It is not pertinent nor desirable to elaborate here on the circumstances that have contributed to the changing concept of species. It is sufficient to recognize that as botanical taxonomy has progressed during the past century the original tenets of the science have been altered by advances in genetics, ecology, physiology, and morphology (16). Neither is it necessary to dwell on the evolution of species concepts and taxonomy in the fungi in general. Most mycologists are well aware of the general problem and many have published their views and opinions as pertaining to the fungi in general or to certain groups. Probably most are agreed, as stated by Bisby (3), that "taxonomy must be as usable, practicable and convenient as possible." This same utilitarian philosophy was earlier expressed by Cunningham (8) who believed that "the object of any taxonomic paper is to present in such a manner as to enable anyone to determine with a reasonable degree of accuracy the species described therein."

It has gradually come to be recognized that the criteria which are valid in delimiting species vary in different groups of organisms (16, 7, 1). This philosophy extends within the various groups of fungi depending on their morphology, life histories, whether parasitic or saprophytic, and other physiological characteristics. Ciferri (7) has given an extended account of the criteria for species determination in fungi and believes that the concept must vary in different subdivisions as we learn more and more about them. Bisby and Ainsworth (4) have expressed the current need in mycological taxonomy: "Many mycologists feel that the taxonomy of fungi is in greater need of synthesis than of more analysis; and a competent study of old genera and species, with much elimination." The greatest problems undoubtedly lie in the parasitic fungi, because for many years the general tendency has been to delimit species on the basis of host, with little or no regard to morphology.

Butler (5) presented a rational review of the subject of morphological versus physiological definition of species of fungi. He effectively rebuked mycologists who have contributed to chaos and confusion by splitting good morphological species entirely or almost entirely on a host specialization basis which results in several or many binomials where there had been one before. He concluded,

> "If we admit that there is no such thing in nature as a pure, invariable, homogeneous species, that habit can modify the facies of a species so long as it endures, that morphological characters are less readily modified by the environment than physiological, and that convenience is often the best guide where to draw the line between different species, we may get some degree of guidance as to which of the tendencies defined above may best be followed. We will be led, I think on the whole to prefer the morphological basis of classification and only in special cases to stress biological differences."

A special committee of the American Phytopathological Society (2), considering problems of nomenclature, agreed that, "This term—(species) should be applied to a group of individuals which can be segregated on the basis of morphological characters of such a nature as to be applicable and determinable by mycologists and pathologists in general and will be available for general, practical taxonomic purposes."

These attitudes toward the taxonomy of fungi in general are applicable to the entire classification of the smut fungi, which stands in need of critical revision. Families, genera, and species are in many instances poorly and often impracticably differentiated. The 2 families of the Ustilaginales (i.e. the Ustilaginaceae and Tilletiaceae) are recognized on such a variable and impracticable basis that more often than not one infers the family relationship of a given smut by analogy. Cunningham (8) considered that only 1 family should be recognized and pointed out that earlier mycologists have proposed the merging of the 2 families. In like manner the validity of some of the genera of smut fungi should be critically reviewed.

But the problems to be considered deal with species concepts of smut fungi. Three approaches have been made to the delimitation of species in this important group of organisms: 1) Morphological; 2) Biological; 3) Biometrical. Repeatedly the discovery of the same or nearly the same morphological species on several hosts has served as a basis for splitting a morphological species into a multiplicity of "species" separated on a host basis and correlated with only minor and insignificant morphological differences if any at all. This practice has been followed chiefly by certain European mycologists. The biometrical delimitation of species has been utilized chiefly by Ciferri (6) who was able to substantiate on this basis many species also recognized on a host basis, and to describe many "new" species. The differences were comparatively small

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(only 1–2μ in the means), even when 200–400 spores were measured. Moreover, the analyses and new species were based on a single collection each and can hardly be interpreted as indicative of more than normal variation.

The wholesale describing of species of smut fungi on a host specialization basis (sometimes demonstrated; more often assumed) has led to a chaotic confusion of invalid species (on morphological grounds) and, therefore, synonymy. Sampson (17) expressed the need for a critical review of the smut fungi on a morphological basis when she said, "To revise the classification of the Ustilaginales, retaining the Linnean binomial only for types clearly distinguishable on morphological characters, would involve a vast amount of labour, but it would be a task which would earn the gratitude of all future mycologists interested in the smut fungi." Later Miss Sampson (18) reemphasized this viewpoint: "In my opinion, weighting each physiologic form with a binomial and an authority only tends to obscure genetic relationships, and does nothing to clarify the species concept." Several other mycologists have expressed a definite preference for a concept of species in the smut fungi based primarily, but not necessarily exclusively, on morphology (4, 10, 12, 13, 19).

Recognizing the importance of host specialization to a moderate degree but still believing emphasis should be placed on morphology, several proposals have been made attempting to reconcile the 2 bases. Ciferri (7) proposed 3 alternatives for straightening out the confused situation and for future guidance: 1) recognize and designate different kinds of "species" (morphological, ecological, pathographic, cultural); 2) recognize and designate species (i.e., good, morphological species), sub-species, varieties, sub-varieties, forms, sub-forms and races; 3) use trinary nomenclature. Other mycologists have expressed a preference for a concept of species in the smut fungi based primarily on morphology but utilizing host specialization only to a reasonable, logical, and practicable degree (1, 5, 8, 17, 18, 19). This latter approach appeals to us, but much is still left to highly variable human judgment in determining just what is "reasonable, logical and practicable."

Savile (19), by implication more than by direct statement, has suggested recognizing host specialization in the smut fungi at the host family level, for purposes of taxonomy. We know that utilizing host specialization at the host species or even the host genus level results, in many instances, in unnecessary and undesirable multiplication of names for the same morphological species. To give absolutely no recognition to host selection by the smut fungi could, and necessarily would, result in consolidation of many species entirely unrelated but of like morphology, due to the comparatively few morphological characters by which they can be distinguished. For instance, one could unite into 1 morphological species 2 such probably unrelated smuts as onion smut and flag smut of wheat and grasses. For purposes of taxonomy, then, and to arrive at a sane and useful concept of species in the smut fungi, and being guided by the expressed opinions, published and unpublished, of mycologists and plant pathologists alike, we believe that species should be delimited primarily on a sound morphological basis but utilizing, at the same time, host selection at the host-family level. Thus, any 2 or more smuts of similar morphology and symptomology, parasitizing different species and genera of the same host family would be considered as belonging to 1 morphological species. If, however, 2 smuts of similar morphology are found on members of different host families, they would be treated as distinct entities (i.e., different species).

Even this species concept is not infallible. Phanerogamic taxonomists are not in complete accord in their family concepts. Thus if 2 morphologically similar smuts are consolidated because they parasitize hosts in the same family but in different subfamilies, and these 2 subfamilies are later given independent family distinction, then the 2 smuts would have to be separated again, or conceivably could result in the recognition of a "new" smut species. But situations like this, while likely to be infrequent, are unavoidable. It has been remarked more than once that taxonomy is dynamic, not static, and persons unable to recognize that this is inevitable but adjustable do not belong in biological fields of investigation. Because of the variability and mutability of living organisms and because of similar variability (and mutability?) of the human mind in its ability to recognize natural relationships and groupings of organisms, and because different concepts are necessary for different groups, it follows that there can be no infallible, easy, and clear-cut system of classification, even when devised specifically for different groups and sub-groups of the fungi.

Assuming a concept of species in the smut fungi based primarily on morphology but taking into account also host specialization at the host family level, the problem still is not completely solved. There remains the issue of a practicable, recognizable degree of morphological variation that may be accepted for purposes of species distinction. Thus one investigator might be content to separate 2 smut species occurring on a single host family on the basis of one having verrucose spores and the other having minutely echinulate spores. Another investigator, recognizing and utilizing more minute differences, might split the verrucose species into 2 or more species on the basis of minute differences in the height, spacing, etc., of the verruculations exhibited in the spores of different collections, or similarly minute variations in spore dimensions. Just as much confusion can result from delimiting species on the basis of morphological differences too minute to be detected and used by any but the extreme specialist as on the basis of too minute a degree of host specialization. Cunningham's utilitarian philosophy (quoted above) of the object of a taxonomic treatise appears sound. Therefore, it would seem unwise to place excess emphasis on the slight

morphological variants so frequently encountered in collections of the same smut on different hosts or on the same host from different geographical or ecological locations. It is not possible, nor would it be desirable, to propose any "rules" specifying what degree of morphological variation is acceptable in delimiting species of smut fungi. To us it seems sufficient to recognize that many species of smut fungi are widespread, have many hosts, and may exhibit considerable morphological variation in different collections. The extremes of this variation may be sufficiently distinct by themselves, but usually they are accompanied by overlapping intergrades that make taxonomic use of these intergrades and the extremes impracticable and frustrating.

It would seem, then, on the basis of the above discussion and the opinions expressed by other mycologists and plant pathologists, that a species concept for smut fungi based on a practicable degree of morphological variation and on host specialization at the host family level would be generally acceptable. This concept necessitates many changes in the taxonomy and nomenclature of the smut fungi. In an attempt to contribute to the stability of the classification of the Ustilaginales, for which Miss Sampson (17) expressed a hope, several consolidations of North American smut fungi are here proposed, together with the synonymy and the valid binominal that would apply under International Rules of Botanical Nomenclature. These consolidations are based on studies of type material in most instances; where type could not be located, authentic specimens were substituted.

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In some of the consolidations proposed above and in certain earlier ones (12), the use of the oldest available specific epithet in conformity with International Rules of Botanical Nomenclature has resulted in a new and seemingly illogical (from the standpoint of derivation of the specific epithet) binomial for an old and well-known smut. These include *Tilletia holci* for bunt on *Anthoxanthum; Urocystis colchici* for onion smut; *Urocystis agropyri* (Preuss) Schroet., for flag smut of wheat; *Ustilago avenae* (Pers.) Rostr., for

black loose smut of barley; and *Ustilago hordei* (Pers.) Lagerh., for covered smut of oats.

Some cereal pathologists have objected (in personal communications) to these "illogical" binomials replacing the binomials by which the cereal smuts concerned have long been designated. In their discussion of the nomenclature of the cereal smut fungi, Stevenson and Johnson (20) point out that the International Rules of Botanical Nomenclature were made official for the United States Department of Agriculture in 1940 and the action applies to all plants including fungi. This of course imposes no obligation on persons outside the United States Department of Agriculture to abide by the Rules, but it sets an example of the philosophy that, since we have the Rules in the first place because a majority of botanists have recognized a need for them, they serve no purpose unless followed.

The claim that it is "illogical" to refer to covered smut of oats as *Ustilago hordei* or to one of the barley smuts as *U. avenae* is untenable on 2 bases: 1) misinterpretation of the significance of these specific epithets, and 2) precedence.

It should be emphasized that the derivation of a specific epithet from a host genus name does not carry with it the necessary implication of pathogenicity *restricted* to that genus any more than such epithets as *canadensis*, *carolianum*, *idahoensis*, *cubense*, *italicum* and the like imply a geographic distribution *limited* to the political units from which such epithets are derived.

The literature in mycology and plant pathology is plentifully supplied with a well established precedent in the use of "illogical" specific epithets. Examples of these follow: Powdery mildew of beans caused by *Erysiphe polygoni* DC.; black crown rot of celery caused by *Centrospora acerina* (Hartig) Newhall; leaf spot of rye caused by *Septoria tritici* Rob. ex. Desm.; damping off of cotton caused by *Rhizoctonia solani* Kühn; stem rot of flax caused by *Sclerotium delphinii* Welch; aster rust caused by *Coleosporium solidaginis* (Schw.) Thüm.; early blight of tomato caused by *Alternaria solani* (Ell. & Mart.) Sor.; scald on barley, caused by *Rhynchosporium secalis* (Oud.) J. J. Davis; downy mildew of ivy (*Parthenocissus* spp.) caused by *Plasmopara viticola* (Berk. & Curt.) Berl. & De T., red leaf of *Rhododendron*, *Arbutus* and *Andromeda* spp., caused by *Exobasidium vaccinii* (Fckl.) Wor.; heart rot of *Larix*, *Pseudotsuga*, *Abies* and *Picea* spp., caused by *Fomes pini* (Thore) Lloyd. Hundreds of such examples could be cited, but the above, selected from different fields of plant pathology, should illustrate the point.

It is probable that part of the general antipathy toward changes in nomenclature stems from an understandable reluctance to unlearn familiar binomials and the necessity to learn new ones. However, a progressive plant scientist should be as eager to learn new nomenclature as new techniques. Both aim toward improvement: one through stability in the nomenclature of plant disease organisms; and the other through improved methods. It does not seem justified to perpetuate errors in nomenclature or deliberately to ignore taxonomic relationships merely to avoid imposing new binomials for old pathogens.

There remains the problem of nomenclature for taxonomic ranks below that of species. Ciferri (7) suggested that the present chaotic situation (in mycology generally) "is not due to the multiplication of systematic units—but to their inadequate taxonomic expression." He believed that 2 courses of action were open: 1) give specific rank and binomials to all systematic units regardless of the criteria for their distinction (i. e. morphological, host selection, cultural, biometrical) but to designate different kinds of species to fit the varying criteria as already reviewed above; 2) give specific rank to morphologic units only, with inferior ranks to designate "units established from criteria other than morphologic." The latter suggestion appeals to us as much the more practical, but Ciferri suggested the use of an impractical number of these inferior ranks (subspecies, variety, sub-variety, form, sub-form, race).

In certain of the rust fungi, particularly the cereal rusts, the nomenclature of these subordinate ranks is now stabilized and well established and provides a pattern that appears equally applicable for the composite species of smut fungi. A good example is in the stem rust of cereals and grasses, *Puccinia graminis* Pers. Here specialized varieties are recognized within the collective, morphological species and are based on host specialization (at the host-genus level) with or without minor morphological variations, and are designated by trinomials (e.g. *P. graminis tritici*). Within the varieties physiologic races are differentiated, again on the basis of host specialization (chiefly by the reaction of agronomic varieties within host species) and these are designated by number (e.g. *P. graminis tritici race* 56). Variants of these races are called biotypes and are designated by letters, as in *P. graminis tritici* race 15-B. It should be emphasized that this classification is based on empirically *demonstrated* host specialization rather than on any assumed specialization based on field observations.

The application of this system of classification to the smut fungi can be illustrated by the cosmopolitan *Ustilago striiformis* (Westend.) Niessl, which causes stripe smut on more than 100 species of grasses. Dozen of synonyms for this smut have arisen during the past half century because of this multiplicity of hosts and the tendency to describe a new "species" of stripe smut every time a new host is encountered. Based on the host specialization studies by Liro (15), Davis (9) and Fischer (11), *U. striiformis* has several varieties already indicated which might be designated as follows:

Ustilago striiformis agrostidis	on *Agrostis*
Ustilago striiformis dactylidis	on *Dactylis*
Ustilago striiformis hordei	on *Agropyron*, *Elymus* and others of the tribe Hordeae

Ustilago striiformis phlei on *Phleum*
Ustilago striiformis poae on *Poa*

It seems safe to predict that in time the existence of other varieties will be demonstrated and, furthermore, that in at least some of the varieties physiologic races will be found. Following the classical example of *Puccinia graminis*, these races could then be designated by number: *U. striiformis phlei* race 4, *U. striiformis hordei* race 7, etc.

A similar example can be cited in the loose smut of cereals and certain grasses. The smut on wheat has long been known as *Ustilago tritici* (Pers.) Rostr. and that on barley as *U. nuda* (Jens.) Rostr. Loose smut on rye has been shown to be morphologically identical to the smut on wheat and barley, and will infect certain wheat varieties (14). Morphologically the same smut as both *U. tritici* and *U. nuda* occurs in the United States on *Agropyron* and *Lolium*. Fischer (12) and earlier workers cited by him have proposed that since the loose smuts of barley and wheat are morphologically inseparable, they be considered as a single species. The binomial *U. nuda* has priority for the morphological species within which the following varieties might be distinguished:

Ustilago nuda hordei on *Hordeum*
Ustilago nuda tritici on *Triticum* and *Secale*
Ustilago nuda agropyri on *Agropyron* (if later demonstrated)
Ustilago nuda lolii on *Lolium* (if later demonstrated)

The system of classification and nomenclature proposed here for the smut fungi, patterned after proven utility in the rusts, should satisfy the various groups of plant scientists in their common need for some means of designating the taxonomic rank with which they are concerned, whether it be morphological species, physiological varieties or physiological races. It allows for a minimum of binomials, and for the mycologist a binomial will, in most cases be sufficient. For those who are concerned with various degrees of host specialization (plant pathologists, plant breeders, and the like), it provides nomenclature to fit their needs.

SUMMARY

Species concepts in various groups of organisms have become more and more divergent as the taxonomy of these groups has progressed through the years. It has gradually come to be recognized that the criteria for species distinction must likewise vary. This philosophy extends within the various groups of fungi, depending on their morphology, life histories, whether parasitic or saprophytic, and other physiological characteristics.

The past practice of describing species of parasitic fungi on a host species basis, with little or no regard to comparative morphology, is deplored. On the other hand, in the smut fungi, where comparatively few morphological characters are available as taxonomic criteria, the adoption of a species concept based exclusively on morphology would result in consolidation of species entirely unrelated but of like morphology.

Therefore, a species concept is proposed for the smut fungi based primarily on a practical degree of morphology but utilizing host specialization at the host family level. Thus, any 2 or more smuts of similar morphology and symptomology, parasitizing different species and genera of the same host family would be considered as belonging to 1 morphological species. However, 2 smuts of similar morphology occurring on members of different host families would be considered as distinct species.

Applying this concept to North American smut fungi, 29 consolidations are proposed, reducing some 46 currently recognized species to synonymy. The legitimate binomial according to International Rules of Botanical Nomenclature is given in each case, together with additional pertinent synonyms.

Some of the consolidations here proposed and certain earlier ones, in conformity with International Rules, have resulted in new and allegedly illogical binomials for old and well-known smuts, e.g. *Urocystis colchici* for onion smut; *Urocystis agropyri* for flag smut of wheat; *Ustilago avenae* for black loose smut of barley; and *U. hordei* for covered smut of oats. Some plant pathologists have considered these binomials illogical on the assumption that when the specific epithet is derived from a host name, then specialization to that host may be inferred. This would seem to be an unwarranted assumption, just the same as such specific epithets as *canadensis, idahoensis, cubense, italicum*, et al. do not imply that geographic distribution is limited to the political units from which they are derived. The literature in mycology and plant pathology is plentifully supplied with well established precedent in the use of "illogical" specific epithets, as illustrated in 12 examples of binomials in current popular usage, selected from several different fields of plant pathology.

The nomenclature for taxonomic ranks below that of species is discussed. It is recommended that the system used in the rust fungi (particularly the cereal rusts), of proven utility, be applied to the smut fungi. This would recognize specialized varieties within collective, morphologic species, based on host specialization (at the host genus level), with or without minor morphological variations, and designated by trinomials. Physiologic races within these varieties would be designated by number. Examples are given of the application of this system to the smut fungi.

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OVERWINTERING OF THREE BACTERIAL PATHOGENS OF SOYBEAN [1]

J. H. Graham [2]

Three bacterial leafspot diseases of soybean occur each year in North Carolina. They are bacterial pustule caused by *Xanthomonas phaseoli* var. *sojensis* (Hedges) Starr and Burkholder, bacterial blight caused by *Pseudomonas glycinea* Coerper, and wildfire caused by *Pseudomonas tabaci* (Wolf and Foster) Stapp.

Little work has been reported on the means of overwintering of *X. phaseoli* var. *sojensis* and *P. glycinea*, particularly in the south, and none on *P. tabaci* in relation to infection of soybeans. Results of studies on the survival of the 3 bacterial leaf spot pathogens in association with seed, soil, and debris are presented in this paper.

REVIEW OF LITERATURE.—Bacterial blight of soybean and its causal agent were first described by Johnson and Coerper (6) in 1917 from Wisconsin. Two years later, Coerper (3) named the causal organism *Bacterium glycineum*. Working independently in North Carolina, Wolf (13) described a disease similar in symptoms to that described by Coerper and named the causal agent *Bacterium sojae*. Most workers now consider the 2 bacterial blight pathogens to be merely different forms of the same species and use *P. glycinea* as the name for the causal agent of bacterial blight.

Kendrick and Gardner (7) in 1920 were the first to show definitely that the pathogen is carried by the seed. Recently, workers at Iowa State College (8) (12) reported that *P. glycinea* could be isolated from seed 16 months old and could remain alive in the soil and on diseased leaves long enough to be an important factor in its survival in Iowa.

The bacterial pustule disease was described by Hedges in 1922 (4). More detailed studies of the disease and its causal agent were reported independently by Hedges (5) and Wolf (14) in 1924. By now, the disease has been found in all important soybean areas of the United States and in Japan.

Lehman (9, 10) showed that the organism would remain alive and pathogenic for at least 9 months in diseased leaves stored in the laboratory and for 3 months in leaves exposed to weathering and decay on the ground. Recently, it was reported from Iowa (8) that the pustule organism was present on and in the seed and could be isolated from seed at least 17 months old. It remained alive in sterile soil held at 10°F. for 69 weeks; however, its pathogenicity was greatly reduced.

Wildfire of soybean was first recognized in 1943. It was then present in North Carolina and probably in most of the soybean-producing areas of the United States. Allington (1) compared isolates from soybean and isolates of *P. tabaci* from tobacco and reported them to be morphologically, physiologically, serologically, and pathologically alike. Prior to this, the soybean had been reported to be susceptible to the tobacco organism only when artificially inoculated (11). Clayton (2) presented evidence which indicated that the organisms from soybean and tobacco were not the same. In field inoculations, he found that the soybean isolate would not infect tobacco plants under conditions that were very favorable for infection by the tobacco isolate. While there has been no study of the means of overwintering of the soybean wildfire isolate, voluminous literature has accumulated on the survival of the tobacco wildfire organism.

OVERWINTERING IN ASSOCIATION WITH SEED.—A field experiment was undertaken to determine the effect of seed treatment on survival of the 3 bacteria in association with seed and the importance of this means of overwintering in relation to subsequent development of the disease.

Three lots of soybean seed harvested from severely diseased plants (as shown in Table 1) and 1 lot from plants showing no symptoms of any bacterial disease were used in this test. The seed was cleaned and ½ of each lot was treated with Arasan (tetramethyl thiuram disulfide) at the rate of 2 oz. per bushel. The plots consisted of 3 12-ft. rows for each treatment and 4 replications were used in each planting. Plantings 1 and 2 were made at Rocky Mount, North Carolina, on April 16 and May 3, respectively, and planting 3 at Plymouth, North Carolina, on May 4.

The percentage of plants showing primary infections of the 3 diseases was calculated from the total number of plants, since seedlings from treated and untreated seed were equally diseased. The data (Table 1) indicate that the 3 pathogens are seed-borne. Seedlings from the 3 seed lots which had been harvested from diseased plants became infected with the respective 3 pathogens, while young plants from the seed lot (F) harvested from plants with no bacterial symptoms showed no evidence of infection arising from seed-borne bacteria.

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TABLE 1.—*Primary infections of bacterial pustule, bacterial blight and wildfire*

Seed			Percentage[a] of plants showing primary infections of		
Symbol	Variety	From plants showing severe symptoms of	Blight	Pustule	Wildfire
D	Ralsoy	Wildfire & pustule	0.32	0.18	0.40
E	Ralsoy	Blight & pustule	0.69	0.20	0.14
H	S100	Wildfire & pustule	0.22	0.29	0.23
F	S100	None	0	0	0

[a] The percentage figures are the average for 3 plantings and were calculated from not less than 3000 plants.

To determine whether or not the small amount of primary inoculum present on these seeds was sufficient to produce widespread infections, disease readings were made throughout the growing season at approximately 2-week intervals. The following rating system, commonly used for rating soybean diseases in North Carolina, was employed:

Per cent of leaves infected per 12 ft. row	Disease rating
less than 1	1
1– 5	2
5– 25	3
25– 50	4
50– 75	5
75–100	6

To present the data graphically, a disease index was calculated for the plants of each seed lot by multiplying the mean disease rating by the mean number of feet of infected plants at each location.

The data on the development of the 3 diseases are presented in graphic form in Fig. 1. Since similar curves were found for the 2 Ralsoy seed lots (D and E), they were combined in each of the graphs.

In the first planting (Fig. 1A), bacterial blight became prevalent during the early part of the growing season. The increase in severity of pustule and wildfire after 14 weeks (early August) was preceded by a period of heavy rainfall (5 in.) with the mean temperature over 25°C. The low temperatures in the spring, incident to early planting, were more favorable for development of blight than wildfire or pustule.

In planting 2 (Fig. 1B), moisture conditions and temperatures became favorable sooner after planting for dissemination and mulitplication of the pustule and wildfire organisms than in planting 1. In late July the average weekly maximum and minimum temperatures increased from 33° to 37°C. and 15° to 21°C., respectively. This increase in temperature coincided with a period of wind and rain storms which disseminated the bacteria throughout the plots. A rapid increase in disease incidence was evident in August.

The early disease development at Plymouth (Fig. 1C) was the result of 4½ in. of rain during the last 2 weeks of May and an increase in the mean temperature from approximately 23° to 30°C.

Thus, the few loci of primary infection arising from bacteria carried in or on the seed were sufficient for the eventual dissemination of the 3 pathogens throughout the plots. Bacterial blight was dominant during the early cool part of the growing season, reaching a maximum about mid-season. By this time many of the infected leaves had abscissed, thus lowering the disease index. As the weather became warmer and

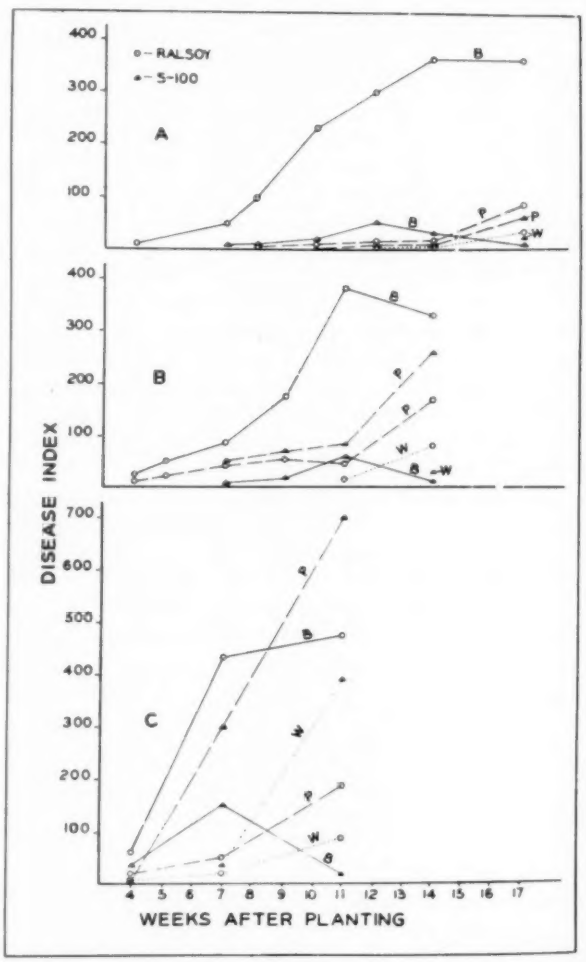

FIG. 1. Development of bacterial blight (B), bacterial pustule (P), and wildfire (W) on two soybean varieties in three plantings: A) planting 1 made April 16 at Rocky Mount; B) planting 2 made May 3 at Rocky Mount; and C) planting 3 made May 4 at Plymouth.

TABLE 2.—*Primary infections of bacterial pustule, bacterial blight, and wildfire on plants grown from seed of various ages*

Lot symbol	Seed		Percentage of plants showing primary infections of		
	Variety	Age	Wildfire	Pustule	Blight
		(months)			
A	Ralsoy	6	0.5	1.1	0
B	46-191	6	0.6	0.8	1.5
C	S-100	6	0.1	0.5	0
D	Ralsoy	18	0	1.1	0
F	Ralsoy	18	0	0.2	0
G	Ralsoy	30	0	0.2	0
H	S-100	30	0	0	0
I	Roanoke	30	0	0	0
J	S-100	6	0	0	0

frequent rains occurred, the pustule disease incidence increased rapidly, followed shortly by a similar increase of wildfire.

It was noted that wildfire symptoms always appeared on plants previously infected with the pustule organism and the incidence of wildfire increased in proportion to pustule infection.

To determine the length of survival of the 3 pathogens in or on soybean seed 8 lots of seed harvested from plants showing symptoms of the 3 diseases and 1 (lot J) from plants grown in an isolated area free of the diseases were planted at Plymouth the following year. Seed, which had been stored in the laboratory for 6, 18, and 30 months, was planted in 12-ft. rows separated by 3 guard rows of a resistant variety (Ogden). The 18-month old seed were from the same lots as used in the previous field test and all 3 pathogens were alive in or on the seed at that time.

As indicated by the appearance of primary infections on the young plants, *X. phaseoli* var. *sojensis* was alive in or on seed of 1 lot even after 30 months of storage (Table 2). The presence of *P. tabaci* and *P. glycinea* was evident only on the 6 month old seed.

PERSISTENCE OF PATHOGENS IN SOIL AND DEBRIS.—Laboratory and field tests were undertaken to obtain information relative to the length of survival of the 3 soybean pathogens in soil and debris. In a laboratory experiment the 3 organisms were added separately to sterile and non-sterile soil and subsequently held in quart jars at 17, 52, and 86 per cent waterholding capacity at 5°, 15°, and 25°C. Diseased leaves mixed with non-sterile soil were also stored under these conditions.

Attempts to recover the bacteria from the soil and leaves were made approximately 1, 2, 3, 6, and 9 months after storage by putting a small sample into tubes of sterile water and Wernham's buffered potato dextrose broth. The water suspensions were stored at 15–19°C. for 2 hours, while the broth cultures were held at the same temperature range for 30–40 hours. Inoculations of plants in the greenhouse were made by gently rubbing the mouth of an inverted culture tube against the dorsal side of the leaf.

The pathogens remained alive and pathogenic in most treatments in sterile soil for the duration of the test; however, in non-sterile soil and in diseased leaves the viability of the bacteria were reduced sharply as the soil moisture and temperature increased (Table 3).

In conjunction with the laboratory test an experiment was set up to determine whether or not the bacteria could survive the winter in soil and debris in the field and produce the disease in the spring.

Thirty-six 8×12 ft. plots separated by sunken alleyways were laid out on a well-drained sandy loam soil in October of 1947. The treatments, randomized and duplicated for each pathogen, were as follows: 1) Broth and water suspensions of each pathogen were sprinkled over the soil and raked in; 2) Broth and water suspensions of bacteria were sprinkled over dried crabgrass hay and partially covered with soil; 3) Diseased leaves were scattered over the plots and held in place by a layer of coarse cheesecloth; 4) Same as 3, except leaves covered with approximately 2 inches of soil; 5) Control, no inoculum added.

In March, samples were taken from each plot to determine whether or not the bacteria were alive. The 3 pathogens were recovered from plots on which diseased leaves had been added and covered with cheesecloth. Only pustule bacteria were viable in the leaves which had been covered with soil. The plots were planted in May with a susceptible variety of soybeans (S-100) which had been harvested from plants free of bacterial diseases.

Only 1 locus of infection appeared in the plots, due probably to the spring and early summer drought. Bacterial pustule developed on a few plants on a plot where diseased leaves had been covered with cheesecloth.

The test was repeated the following year with the addition of 6 plots of wheat. In October, the plots were sprinkled with the bacterial suspensions after

TABLE 3.—*Length of survival of 3 pathogens in soil and diseased leaves under various conditions of storage*

Temperature	Soil moisture	Last month bacteria recovered as indicated by infection of test plants [a]								
		Sterile soil			Non-sterile soil			Diseased leaves in non-sterile soil		
C.		BP	BB	Wf	BP	BB	Wf	BP	BB	Wf
5	D [b]	9	9	9	6	6	0	9	3	2
	M	9	9	9	6	9	2	9	1	2
	W	9	9	6	2	2	1	2	3	1
15	D	9	9	9	3	9	2	6	3	6
	M	9	9	9	3	2	1	6	2	1
	W	9	9	6	2	3	1	6	1	0
25	D	9	9	9	3	0	2	1	1	1
	M	9	9	9	1	0	0	1	0	0
	W	9	6	3	1	0	0	0	1	0

[a] Isolations made 1, 2, 3, 6 and 9 months after storing.
[b] D—17% of water-holding capacity.
M—52% of water-holding capacity.
W—86% of water-holding capacity.

broadcasting the wheat and again when the seedlings had emerged. All plots were planted with S-100 soybean seed in the spring and observations made during the growing season for loci of primary infection.

Plants on 3 of 6 plots having leaves not covered with soil became infected with *X. phaseoli* var. *sojensis*. Two of these had been covered with leaves heavily infected with this pathogen and the other plot with leaves showing severe infection of *P. tabaci* in addition to some pustular lesions. Approximately 1 week after the above symptoms were observed, wildfire lesions appeared in the latter plot on several of the leaves showing pustule lesions. Plants on 2 of the plots where the leaves infected with the pustule pathogen had been covered with soil also showed primary pustular lesions.

No bacterial blight lesions were found on the plants in any of the plots covered with infected leaves. None of the plants on the plots in which the soil, hay, and wheat were inoculated with the 3 bacterial suspensions became infected.

The laboratory test was confirmed by the field tests which indicated that the bacterial blight, pustule, and wildfire organisms can survive the winter in leaves on soil and that the pustule and wildfire organisms can initiate infection on young soybean plants grown in this soil the following spring. In all tests, the pustule organism showed a greater capacity to persist in leaves that did the other 2 pathogens. The indications obtained in the laboratory test that *X. phaseoli* var. *sojensis* and *P. glycinea* might overwinter as free bacteria in the soil were not confirmed by the field studies. Either the bacteria did not survive free in the soil in the field or the population was too small to be recovered by the method employed.

SUMMARY

The 3 soybean pathogens, *Xanthomonas phaseoli* var. *sojensis*, *Pseudomonas glycinea*, and *Pseudomonas tabaci* which cause bacterial pustule, bacterial blight, and wildfire, respectively, were found to be seed-borne. Although the percentage of initial infection on seedlings from infected seed was low, it was sufficient to produce moderately severe disease when weather conditions were favorable.

Seed treatment with Arasan did not reduce the amount of primary bacterial infection.

P. tabaci and *P. glycinea* were detected in seed 6 months after harvest, but not in seed 18 months old. *X. phaseoli* var. *sojensis* was found in seed 6, 18, and 30 months after harvest.

In a laboratory test, the 3 pathogens persisted in sterile soil for at least 9 months. *X. phaseoli* var. *sojensis* and *P. glycinea* survived in field soil and in diseased soybean leaves in field soil long enough to indicate this as a possible medium of overwintering.

In field experiments, the 3 pathogens persisted in diseased soybean leaves on the soil surface throughout the winter. *X. phaseoli* var. *sojensis* and *P. tabaci* from these leaves infected young soybean plants the following spring. The 3 organisms could not be recovered from soil or hay inoculated with bacterial suspensions in the field experiments.

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CULTURAL AND EPIPHYTOTIC RELATIONSHIPS OF THREE BACTERIAL PATHOGENS OF SOYBEANS [1]

J. H. Graham [2]

During an earlier study,[3] certain relationships were observed in the occurrence of 3 bacterial leafspot diseases of soybeans, bacterial pustule caused by *Xanthomonas phaseoli* var. *sojensis* (Hedges) Starr and Burkholder, bacterial blight caused by *Pseudomonas glycinea* Coerper, and wildfire caused by *Pseudomonas tabaci* (Wolf and Foster) Stapp. A number of experiments designed to gain a better understanding of the associations are here reported.

ASSOCIATION OF PATHOGENS.—In the field it was observed that wildfire appeared first on plants in areas previously infected with the bacterial pustule pathogen. Closer examination revealed symptoms of both diseases on the same leaves and often 1 or more pustules were found in the centers of the wildfire lesions. Three experiments were initiated to determine whether or not the wildfire bacteria enter the leaf tissue through bacterial pustule or through blight lesions.

Isolations were made from various types of young bacterial lesions on soybean leaves. Lesions considered to be typical of 1 of the 3 organisms often contained 1 of the other pathogens. The pustule organism was isolated from most of the wildfire lesions, in some instances even in the absence of visible pustules. The wildfire organism alone was isolated from only 4 of 15 wildfire lesions; all others contained wildfire and pustule bacteria. In 1 instance, blight and pustule bacteria were isolated from a lesion which had some characteristics of both diseases.

Further explanation of this association was obtained in field observations. Three sections of a field nursery were inoculated separately with the pustule, blight, and wildfire organisms. Moderate pustule and blight infection was obtained, but no wildfire symptoms appeared on the plants inoculated with that organism. In an adjacent field, plants which were inoculated with a mixture of the bacterial pustule and wildfire bacteria developed symptoms of both diseases. This indicated that lesions of the pustule organism serve as points of entrance for the wildfire pathogen.

In greenhouse experiments it was repeatedly shown that good wildfire infection could be obtained by lightly spraying leaves having bacterial pustule lesions, whereas wildfire infection was not obtained by spraying healthy leaves. In similar experiments, infection was seldom obtained when the wildfire organism was sprayed on leaves with bacterial blight lesions.

Clayton[4] recently showed in field inoculations (without wounding the plants) that the soybean isolate from wildfire lesions would rarely infect soybean plants under the same conditions that the tobacco isolate produced good infection on tobacco. This supports the evidence in the present paper that the soybean isolate of *P. tabaci* infects field-grown soybean plants chiefly through injuries. Although *P. tabaci* can enter through mechanical injuries, bacterial pustule lesions are probably the main gateway. It is postulated that *P. tabaci* (from soybean) is not sufficiently virulent to initiate infection in healthy soybean tissue under natural conditions. The wildfire pathogen is presumed to produce a toxic substance injurious to host cells in advance of the bacteria because bacteria can be isolated only from the necrotic area and not from the surrounding chlorotic zone. Wildfire bacteria entering healthy soybean leaf tissue apparently do not encounter conditions favorable for growth and production of this toxic substance. After the pustule bacteria have invaded and killed some of the plant cells there is apparently a favorable medium for development of the wildfire bacteria. In nature, dews and rains wet the ruptured pustules, thereby providing means of entrance for the wildfire bacteria.

These relationships between the pustule and wildfire diseases may be of significance in development and practice of control measures since any practices which control bacterial pustule disease will indirectly also control wildfire. Field observations also indicate that varieties resistant to pustule are also resistant to wildfire.

MANIFESTATIONS OF AN ANTAGONISTIC SUBSTANCE.—Evidence of an antagonistic action was obtained when isolating the 3 pathogens from debris and soil by use of broth media; often only the wildfire organism could be recovered when all 3 should have been present. To determine whether or not *P. tabaci* produces a substance antagonistic to *X. phaseoli* var. *sojensis* and *P. glycinea*, test tubes of beef dextrose broth were inocu-

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TABLE 1.—*Reproduction rate of Xanthomonas phaseoli var. sojensis and Pseudomonas tabaci incubated separately and together*

Time after mixing (hours)	Millions of cells of X. Phas. soj. when grown:		Millions of cells of P. tabaci when grown:	
	Alone	With P. tabaci.	With X. phas. soj.	Alone
0	320	230	80	33
3	176	29	51	25
6	145	24	21	35
9	230	71	40	51
12	400	10	60	55
24	1000	10	1100	1280
48	---	0	3400	---

lated with the 3 pathogens separately and in various combinations.

Plants inoculated with 2-day old broth cultures of the wildfire organism in combination with pustule or blight bacteria produced only wildfire symptoms and only the latter could be recovered from the infected leaves. Mixtures of pustule and blight organisms caused symptoms of both diseases on plants in the greenhouse. When the pathogens were incubated separately, then mixed and immediately sprayed onto plants, all organisms produced typical symptoms, though the pustule and blight disease incidence was lower than when inoculated separately.

Plate dilutions were made from separate cultures and from mixtures of pustule and wildfire bacteria after 3, 6, 9, 12, 24, and 48 hours. Counts presented in Table 1 are transformations made from counts on the higher dilutions. The pathogens were easily recognized in the dilution plates since colonies of *X. phaseoli* var. *sojensis* are yellow and those of *P. tabaci* are white.

After 24 hours the number of bacterial cells of *X. phaseoli* var. *sojensis* growing alone and *P. tabaci* growing alone and in mixture had increased greatly, while the former in mixture with *P. tabaci* had decreased. After 48 hours, *X. phaseoli* var. *sojensis* could not be recovered from the mixture. When the test was made with approximately equal numbers of both organisms in the stock culture, the pustule bacteria were killed after 22 hours.

The preceding experiments show that *X. phaseoli* var. *sojensis* cannot survive for even 2 days when grown with *P. tabaci* in broth media. Since preliminary investigations indicated that a food relationship or a change in pH was not the cause, tests for a diffusible antagonistic substance were made.

Bacterial suspensions of *X. phaseoli* var. *sojensis* or *P. glycinea* were mixed with liquid agar and poured into petri dishes. After solidification, a sterile filter paper disc approximately 15 mm. in diameter which had been dipped in a broth culture of the wildfire organism was placed on the agar.[5] After 2 days, little or no growth of the pustule or blight organisms occurred near the discs, leaving a clear, apparently bacteria-free zone ranging from a few mm. to 20 mm. in diameter. The clear zones in the plates seeded with *X. phaseoli* var. *sojensis* were usually slightly larger than in the *P. glycinea* plates. Similar inhibition zones were produced by isolates of *P. tabaci* from tobacco.

This phenomenon was also demonstrated by seeding agar discs with the blight or pustule organisms and streaking the wildfire bacteria on the opposite side. Little or no growth of *P. glycinea* or *X. phaseoli* var. *sojensis* appeared under the streaks, while abundant growth occurred at a distance.

Attempts were made to separate the diffusible substance from the bacterial cells with a Seitz filter. No indication of the substance was found in the sterile filtrate even when reduced 90 per cent in volume under vacuum. Negative results with the filter paper disc method were also obtained in attempts to precipitate the antagonistic agent with alcohol.

DISCUSSION.—The relationships of the 3 leaf-spot pathogens of soybean are unique. *X. phaseoli* var. *sojensis* and *P. glycinea* cannot be recovered when grown 48 or more hours with *P. tabaci* in broth cultures. The inactivation of pustule and blight bacteria is apparently not due to a change in reaction or a food relationship since the antagonized bacteria grow well in the bacteria-free filtrate of *P. tabaci*. A diffusible substance is produced by this organism, but it could not be separated from the bacteria. This antagonism is not caused by the toxic substance which produces the chlorotic characteristic of wildfire lesions on leaf tissue since Clayton[6] showed that the latter substance is heat-stable and filterable.

SUMMARY

Numerous examples of the association of *X. phaseoli* var. *sojensis* and *P. tabaci* are cited. Pustule lesions were found on almost all leaves showing wildfire symptoms. *X. phaseoli* var. *sojensis* was isolated from most of the young typical wildfire lesions examined.

P. tabaci infects soybean leaves primarily through pustule lesions.

In culture *P. tabaci* produces a diffusible substance antagonistic to *X. phaseoli* var. *sojensis* and *P. glycinea*. The antagonistic substance could not be separated from the bacteria by the methods used.

Tobacco isolates of *P. tabaci* were similarly antagonistic to *X. phaseoli* var. *sojensis* and *P. glycinea*.

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THE PHYSIOLOGY OF LYCOMARASMIN PRODUCTION BY FUSARIUM OXYSPORUM F. LYCOPERSICI [1]

A. E. Dimond and P. E. Waggoner

Lycomarasmin is a metabolic product of *Fusarium oxysporum* f. *lycopersici* which produces necrosis of interveinal cells or desiccation of marginal cells in leaflets when absorbed by tomato cuttings (3). The role of lycomarasmin in Fusarium wilt of tomatoes is not yet known. Thus it has never been isolated from the diseased plant. Although the production of lycomarasmin *in vitro* is well established (8, 9, 10, 16) all workers agree that it can be isolated only from culture filtrates which are at least 2–4 months old.

If lycomarasmin is found only in old cultures, the physiology of its production in culture may be of interest in indicating its role in the diseased plant. In the present study the consumption of reducing sugars and inorganic nitrogen by *F. oxysporum* f. *lycopersici* was followed over a 103 day period and related to pH changes and mycelial growth as measured by dry weight. The appearance of lycomarasmin and of polysaccharides was also related to the mycelial growth curve.

MATERIALS AND METHODS.—In these studies the R-5-6 strain of *Fusarium oxysporum* f. *lycopersici* of Wellman (15) was employed because it was a virulent strain, known to produce lycomarasmin (3). It was grown on the modified Richard's medium used by Plattner and Clauson-Kaas for lycomarasmin production (8). Erlenmeyer flasks of 500 ml. capacity, containing 100 ml. of nutrient were inoculated and maintained in quiet culture at 25° C. Culture filtrates were analyzed from time to time over a 103 day incubation period. In the process the mycelial mat was removed by filtration and the mats were weighed after drying for 18 hours at 85° C. Volume of the culture filtrate was measured, the pH determined, and then aliquots were withdrawn as suitable for the analysis in question.

All chemical analyses of the culture filtrate were made by standard methods which will not be described here in detail. Reducing sugars were estimated by Somogyi's micromethod (11). Polysaccharides were estimated by obtaining the difference in reducing sugar levels before and after a 2-hour acid hydrolysis, as obtained by Somogyi's method. For the acid hydrolysis a volume of culture filtrate containing the requisite amount of reducing sugars was pipetted into a 1 × 6 in. test tube. The sample was acidified in accordance with the standard method for estimating polysaccharides (1) and was digested, using a capillary reflux condenser, devised by Vickery et al for microdetermination of amide nitrogen (13). Following digestion, the sample was neutralized with alkali and the analysis for reducing sugars was repeated.

Ammonium-N was separated from the culture filtrate by the permutit method and estimated as ammonia with Nessler's reagent (6). Nitrate-N was determined by the use of the phenol disulfonic acid method (6). For components of the culture filtrate initially present in the nutrient medium, i.e., for nitrate-N, ammonium-N, and reducing sugars, the concentrations in mg./ml. were corrected for loss by evaporation from the culture during the growth period. However, polysaccharides were not present initially and are reported as mg. of glucose per ml. without correction for concentration by evaporation because they are of interest in terms of the toxicity they produce in tomato cuttings.

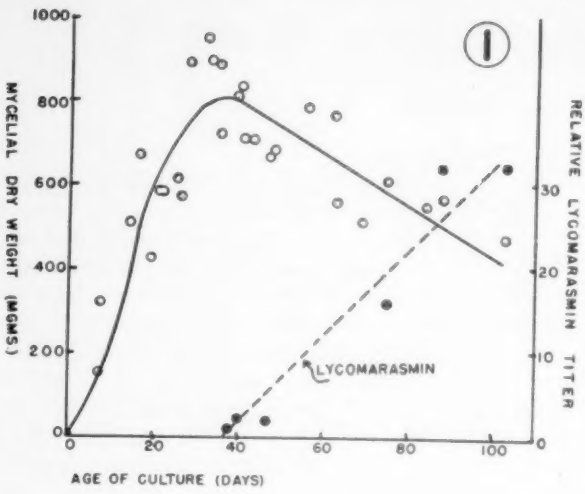
Samples of culture filtrates were assayed for lycomarasmin content by standing tomato cuttings in them and noting whether the symptoms characteristic of lycomarasmin developed. These symptoms were compared with those produced by a sample of purified lycomarasmin [2] (m.p. 228° C.). Because some of the symptoms produced by polysaccharides can be confused with those caused by lycomarasmin, 2 further properties of lycomarasmin were used to identify it in culture filtrates. These are its 10-fold potentiation by iron ion (3) and its inactivation by heating (8). Accordingly, for every culture filtrate 3 tests were made, each in a dilution series: 1) untreated filtrate, 2) filtrate plus ferrous sulfate (0.0005 M) and 3) heated filtrate plus ferrous sulfate (0.0005 M). Lycomarasmin was considered to be present in culture filtrates when toxicity was increased by presence of ferrous ion and decreased by heating the filtrate for ½ hour at 80° C. The relative concentration of lycomarasmin was estimated through the dilution end-point, the highest dilution of the culture filtrate in which lycomarasmin could be demonstrated. The dilution end-point of the unheated filtrate to which ferrous sulfate had been added was used as the basis of the estimate. Because lycomarasmin is unstable (14) results of assays depend upon the time required for absorption of a minimum dose unless this exposure time is a constant. In these experiments exposure time was held constant in assays at 24 hours.

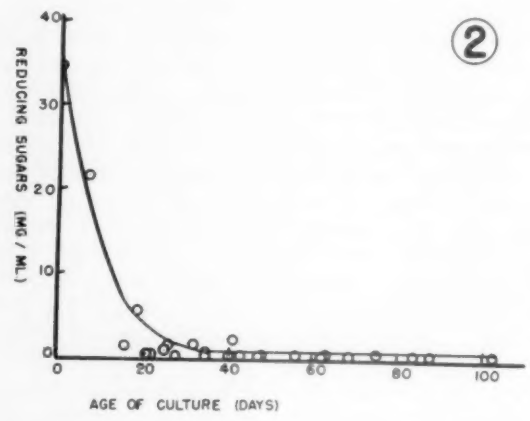
Analyses were always made on cultures of 3 ages on 1 day. Biological variation within cultures was estimated by making duplicate analyses. By these procedures and by employing chemical standards in all analyses, variation resulting from chemical errors and biological variation was minimized or estimable. Values reported represent the means of duplicate analyses.

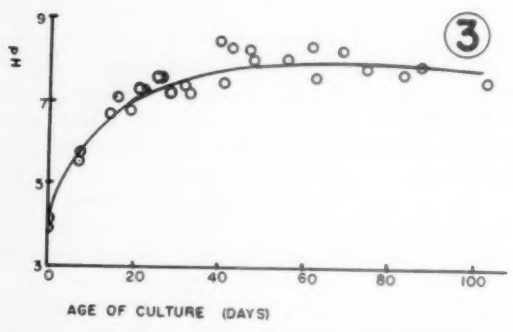
RESULTS.—The growth curve of *Fusarium oxysporum* f. *lycopersici* on the modified Richards nutrient indicates that maximum mycelial weight is attained in

[1] Accepted for publication December 19, 1952.

[2] The sample was supplied by Dr. E. Gaumann.







FIGS. 1–3. Fig. 1. The growth curve of *F. oxysporum* f. *lycopersici* on modified Richard's medium in quiet culture and the appearance of lycomarasmin in the culture filtrate as a function of time. Linear segments of both curves have been fitted.—Fig. 2. Consumption of glucose from modified Richard's nutrient by *F. oxysporum* f. *lycopersici* as a function of time.—Fig. 3. Shift in hydrogen ion concentration of modified Richard's nutrient as a function of time.

about 30 days (Fig. 1). Thereafter there is a constant decrease in mat weight as lysis becomes evident. A line fitted to the portion of the growth curve in which lysis is evident indicates that the rate of mat loss was 5.45 ± 0.89 mg. per day.

In contrast, lycomarasmin did not become detectable in the culture filtrate until the maximum mat weight had already been attained and lysis of the mat was evident (Fig. 1). After appearing, the relative titer of lycomarasmin rose steadily at least until the 85th day.

Reducing sugars dropped rapidly from their initial level and became essentially exhausted in 30 days (Fig. 2). There was no change in reducing sugar levels after the 40th day, values remaining about 0.25 mg./ml. until the experiment was completed. It is clear from the pH curve of the culture filtrate (Fig. 3) that there was no significant amount of acid production as a result of glucose utilization. The hydrogen-ion concentration (Fig. 3) rose steadily during the period of rapid growth and carbohydrate consumption, and attained a value of about pH 7.8 within the first 40 days of growth. Thereafter pH remained essentially constant for the duration of the experiment.

The modified Richard's solution contained ammonium nitrate as the sole nitrogen source. Analyses indicated that nitrate-N disappeared rapidly at first and that by the end of the fourth week, nitrate-N was no longer being utilized (Table 1). At this time it was still present in concentrations of approximately 0.25 mg./ml. In contrast, ammonium-N was utilized more slowly at first and attained a constant level after 4 weeks which was somewhat higher than was true of nitrate-N.

In the absence of acid production by the fungus, the nitrogen consumption pattern of *Fusarium oxysporum* f. *lycopersici* (Table 1) would account for the properties of the pH curve (Fig. 3). When an organism can use either nitrate- or ammonium-N, selective absorption of nitrate ion generally results in an increase in pH as the remaining cation becames associated with hydroxyl

TABLE 1.—*The disappearance of inorganic nitrogen and appearance of polysaccharides in culture filtrates of Fusarium oxysporum F. lycopersici*

Age of culture days	NO_3-N mg./ml.[a]	NH_4-N mg./ml.[b]	Polysaccharide mg./ml.[b]
0	1.39	1.13	0.00
7	0.87	1.13	0.00
14	0.21	1.00	[c]
21	0.29	0.90	0.31
28	0.22	0.89	0.33
35	0.37	0.81	0.12
43	0.17	0.65	0.75
47	0.24	0.69	0.92
56	0.25	0.56	0.99
62	0.23	0.71	1.14
75	0.22	0.53	0.78
84	[c]	[c]	0.79
88	[c]	[c]	1.02
103	[c]	[c]	0.92

[a] Corrected for loss by evaporation to concentration in initial volume.
[b] Net concentration per ml., expressed as mg. of reducing sugars per ml. of culture filtrate.
[c] Not determined.

ion. During the early phase of growth, nitrate was absorbed from the nutrient medium more rapidly than ammonium ion and pH increased as a result. Later ammonium ion was absorbed simultaneously but less rapidly than nitrate ion. During this period the increase in pH with time was less rapid than formerly. Finally, as carbohydrates became depleted in the nutrient, conversion of inorganic to organic N ceased, as did absorption of inorganic N from the nutrient. Thereafter pH remained essentially constant. At this stage a greater proportion of the nitrates than of ammonium ion initially available had disappeared from solution. Thus the general properties of the pH curve can be accounted for.

Polysaccharides were detectable at the end of the third week and increased in concentration during the following weeks until concentrations in excess of 1 mg./ml. were observed (Table 1). Polysaccharide concentration did not continue to rise indefinitely, however, but levelled off after the period of active growth had passed.

Polysaccharides have been shown to cause marginal desiccation of tomato cuttings (7) and have previously been isolated from culture filtrates of *Fusarium* species (12). In view of such findings it is worthwhile to compute whether the amount of polysaccharide present in culture filtrates in the present experiment could cause damage to tomato cuttings which absorb the filtrate. Gaumann and Jaag (4) have determined the effects of inulin, a polysaccharide, on tomato cuttings, and have noted that the minimum toxic dose is approximately 1500 mg. inulin per kg. fresh weight of cutting. For a small cutting, weighing 1 gm., only 1.5 mg. inulin are required to produce injury. In the present experiments polysaccharides were determined, after they had reached a constant level, at concentrations of 0.9 mg./ml. Accordingly, a toxic dose of polysaccharide will be absorbed by a small cutting, weighing 1 gm. and taking up only slightly more than 1 ml. of culture filtrate. Quite evidently caution must be exercised in assays for lycomarasmin where polysaccharides may also be present, because these two can invoke similar symptoms in cuttings. Because lycomarasmin is activated by iron and inactivated by slight heating, it can be distinguished in culture filtrates from polysaccharides which are unaffected by such treatment.

Discussion.—It is now necessary to relate to one another the observations on the change with time in the levels of the various components of the culture filtrate before discussing the role of lycomarasmin in Fusarium wilt of tomato. The mycelial weight attained its maximum value within 35 days, the period of active growth (Fig. 1). During this period, all the components of the culture filtrate under examination excepting lycomarasmin also showed their principal change. Reducing sugars became depleted within this period (Fig. 2). Ammonium- and nitrate-N decreased to their minimum levels (Table 1). Polysaccharides appeared and rose almost to their maximum level (Table 1). The hydrogen-ion concentration decreased to alkaline levels during this same period (Fig. 3).

By the 40th day after inoculation, conditions in the culture filtrate had become fairly well stabilized. Reducing sugars were depleted (Fig. 2). pH had become a constant at a value of 7.8 (Fig. 3). Nitrate- and ammonium-N were no longer being consumed (Table 1), owing to depletion of glucose in the nutrient. Polysaccharide formation had just about ceased (Table 1). Mycelial weight from this point on decreased as lysis became evident (Fig. 1).

In contrast, lycomarasmin made its first appearance in the culture filtrate after lysis was clearly evident in the mycelial mat (Fig. 1). It is probable that lysis is a process which begins rather early during the growth of the mycelium, but which becomes evident in terms of mat weight only after growth of the mat ceases. If so, lysis had been under way for a considerable period before lycomarasmin first appeared in the culture filtrate. Other toxic products of fungus metabolism are known to be produced under similar conditions. Thus streptomycin does not appear in the culture medium until *Streptomyces griseus* approaches its maximum growth. The peak of streptomycin production occurs considerably after the peak of mycelial weight (5).

Pertinent to the apparent rate at which lycomarasmin appears in culture filtrates is the instability of lycomarasmin. On heating, lycomarasmin splits off NH_3 and becomes inactive (8). Recently the heat of activation and the rate at which lycomarasmin becomes inactivated at 29° C. have been studied (14). Its half-life (the time for half of the molecules to decompose) has been estimated at 4.8 days in aqueous solution at a pH of 3.6. However, at the H-ion concentration of the fluid in vessel elements in the tomato plant, pH 6.0, lycomarasmin, while it decomposes at a measureable rate, is considerably more stable. The amount of breakdown depends upon the amount present. Therefore, the more lycomarasmin in the culture filtrate, the more it disappears through decomposition. Thus the total amount of lycomarasmin formed must be somewhat higher than the titer at any given time would indicate (Fig. 1). The total lycomarasmin synthesized appears to be no more than 5 to 10 times the quantity of lycomarasmin detectable in the filtrate (14), this estimate being based on the instability of lycomarasmin at pH 3.6. Estimates of lycomarasmin decomposition at higher H-ion concentrations result in lower estimates of lycomarasmin production. Hence, using the stability of lycomarasmin at pH 3.6 as a basis for the estimate favors the hypothesis of wilt production by lycomarasmin to the greatest possible extent.

On Richard's medium the pH initially was 4.0 and it rose during the first 10 days to a value of 6.0, the pH of tracheal fluids in the tomato. Lycomarasmin is more unstable at an acid H-ion concentration than at neutrality. It might be argued that the non-appearance of lycomarasmin in culture filtrates resulted from rapid decomposition at acid pH values and slower decomposition as the pH of the nutrient medium approached 6.0. However, the non-appearance of lycomarasmin

between the 10th and 40th days cannot have resulted from this effect.

It will be useful now to compute the actual quantities of lycomarasmin in solution, detected by a cutting response (Fig. 1). Gaumann has estimated the minimum toxic dose of lycomarasmin as ranging from 10–15 mg./kg. fresh weight of tomato cuttings when lycomarasmin is activated by iron whereas in the iron-free state it is 100–150 mg./kg. (3). To be on the conservative side, let us assume that the lycomarasmin was iron activated in these studies and that the lower estimate of minimum toxic dose is correct. Then, for a cutting of 1 gm. weight, absorbing lycomarasmin within 24 hours, the minimum toxic dose would be .01 mg. of lycomarasmin. If the cutting absorbs 5 ml. of culture filtrate during this period, then the titer of lycomarasmin will be .002 mg./ml. Thus, 0.002 mg./ml. of lycomarasmin would be the minimum detectable in the bioassay. Since lycomarasmin was detected in filtrates diluted 32 times at the end of 103 days, the maximum titer would be 0.064 mg./ml. A factor of 10 will adjust these readings for the situation where lycomarasmin is introduced into the cuttings in the iron-free state.

In the present study, the appearance of 2 metabolic products has been followed: polysaccharide and lycomarasmin. Polysaccharide was detected in the culture filtrate by the end of the third week whereas lycomarasmin was not noted until the end of the sixth week. Polysaccharide appeared while the fungus was growing rapidly and levelled off after growth ceased. Lycomarasmin appeared only after the period of rapid growth had ceased and lysis of the mycelium was evident; it continued to rise in titer in the culture filtrate for the duration of the experiment.

The least detectable amount of polysaccharide, by the method employed, was 0.05 mg./ml. of culture filtrate. As has been indicated previously, the minimum amount of lycomarasmin detectable by tomato cuttings, assuming iron activation, was 0.002 mg./ml. of culture filtrate. The bioassay for lycomarasmin, therefore, was more sensitive than the analytical method for polysaccharaide. If lycomarasmin was not detected during the period of active growth, its production was negligible. Although decomposition of lycomarasmin would reuire a correction of the production curve in late stages of the study, it would not be important in the very early phases of production because the amount decomposing depends upon the amount already present. Therefore, this correction becomes important only as lycomarasmin accumulates in the culture filtrate.

The bioassay for lycomarasmin is in terms of the response of tomato tissue, the same tissue involved in wilting response in the infected plant. Therefore, if the amount of lycomarasmin in the culture filtrate is less than can be detected by cuttings, it can be ignored insofar as wilt production *in vivo* is concerned.

A comparison of the appearance of symptoms of Fusarium wilt in the inoculated tomato plant, as a function of time, with the appearance of lycomarasmin in culture filtrates is significant. A tomato plant will show wilting symptoms of Fusarium wilt within 2 weeks of inoculation. Frequently, in an additional 30 days (45 after inoculation) the plant is dead. In terms of lycomarasmin as a vivotoxin this picture suggests that lycomarasmin production *in vivo* must commence earlier than it was found to occur *in vitro* and that the titer must be higher than was observed at the end of 45 days. What determined the actual time when lycomarasmin appeared in these studies was the volume and surface relations of the culture and medium, and the composition of the nutrient medium. Thus the sooner nutrients reached depletion, the sooner lycomarasmin appeared. It may be argued, however, that nutrients are not depleted in the plant within the time for symptoms to appear and that the large production of lycomarasmin necessary to injure the plant by the small amount of mycelium in the roots would require a much higher production rate than was observed in these studies.

If lycomarasmin is a lytic product *in vivo* as well as *in vitro*, and if the wilting of foliage is caused by lycomarasmin, one may consider where lysis is likely to occur in the diseased plant within 15 days of initial infection. Probably the point of initial infection in the root would be most likely to be depleted of nutrients; this would be a relatively small part of the root as a whole. Since lycomarasmin does not act on vascular tissue, but rather on parenchyma in the leaf blade, it must be translocated from the roots to the leaves to become active. In this process it would hardly be translocated selectively to the oldest leaves first and then to young ones in the manner that symptoms appear in the diseased tomato plant (10), but rather would be distributed uniformly to all leaves. Experiments on cuttings with lycomarasmin indicate that young leaves are no more sensitive to lycomarasmin than are old ones. The pattern by which wilting symptoms appear on the plant seems too definite to be compatible with their being caused by lycomarasmin if it is a lytic product of the mycelium.

Finally, in Fusarium wilt there is a very high correlation between the distribution of vascular discoloration in the stem and the severity of wilting symptoms (2). A leaf generally does not show symptoms until the fungus, advancing from the root up the stem, is in the vicinity of the node to which that leaf is attached. The mycelium adjacent represents the most recent growth of the fungus in its advance up the stem and could not be undergoing lysis. It is difficult, therefore, to harmonize a role of lycomarasmin as a vivotoxin with the correlation between the pattern of wilting symptoms and the distribution of fungus in the stem.

SUMMARY

The physiology of lycomarasmin production has been studied, using the R-5-6 strain of *Fusarium oxysporum* f. *lycopersici*. A modified Richard's solution, known to favor lycomarasmin production, was inoculated and

the culture filtrate was analyzed for levels of sugars, nitrate-N, ammonia-N, pH, mycelial dry weight, polysaccharide content, and lycomarasmin. Lycomarasmin was estimated by the response of tomato cuttings and was identified through its known activation by iron and inactivation by heating.

Maximum mycelial weight was attained within the first 6 weeks; within this same period, reducing sugars became depleted from the medium, while nitrate- and ammonia-N were utilized as far as possible without addition of further carbohydrate. pH also became stabilized in this period as did polysaccharide levels. However, lycomarasmin toxicity was first noted at the end of the sixth week and the titer of lycomarasmin then rose as weight of the mycelial mat fell at the rate of 5.45 mg. per day. These findings suggest that lycomarasmin is a product of lysis rather than a product of rapidly growing mycelium. It is noted that foliar symptoms of Fusarium wilt in naturally infected plants appear sooner than would be likely if the symptoms arose from a toxin which is of lytic origin.

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FURTHER STUDIES ON HOST SPECIALIZATION IN THE HEAD SMUT OF GRASSES, USTILAGO BULLATA [1]

Jack P. Meiners and George W. Fischer

Physiologic specialization in head smut of grasses, *Ustilago bullata,* was reported by Fischer in 1940 (2). Eight races were identified by the differential reaction of 14 species of grasses. This problem has since been further investigated and 4 additional races identified. In this study, inconsistent reactions of certain collections of head smut presented problems in race classification. An attempt has been made to determine the reasons for this variable performance. This paper presents the results of the investigation, along with descriptions of the newly identified races.

MATERIALS AND METHODS.—One hundred forty-one collections of head smut were used in the study. They were obtained from 41 species of the genera Agropyron, Bromus, Elymus, Festuca, Hordeum, and Sitanion. The collections, made in 18 states and territories of the United States and 2 foreign countries[2], included those used previously by Fischer (2) and 97 new ones.

Two kinds of inoculum were used. The basic inoculum for all collections was smut spores. For comparative purposes, paired monosporidial lines from a single spore of certain collections and races were used. Eleven grass species were used for race differentiation, 3 less than the number reported in the previous paper (2). The current set of differential species now includes: *Bromus carinatus* Hook. and Arn. (P-2725),[3] *B. catharticus Vahl* (F-161), *B. hordeaceus* L. (F-155), *B. japonicus* Thunb. (F-154), *B. mollis* L. (F-194), *B. secalinus* L. (F-329), *B. tectorum* L. (F-165), *Elymus canadensis* L. (P-2389), *E. glaucus* Buckl. (P-1851), *E. sibiricus* L. (P-225) and *Hordeum nodosum* L. (P-2723).

Prior to inoculation of the seed, natural contamination was eliminated by soaking the seed in formaldehyde solution (1-320) for 1 hour. The treated seed was washed in running water to remove the formaldehyde and then allowed to dry. Inoculation was performed by the partial vacuum method, and the inoculated seed was handled in the manner used earlier by Fischer (2).

Host reaction to collections and races was determined by the percentage of smut in each row, based on head count.

EXPERIMENTAL RESULTS.—*Race identification.*—Four new races identified in this study are designated as Races 9, 10, 11, and 12. The reaction of the differential species of grasses to collections of head smut representing these new races is in Table 1.

Race 9, represented by 1 collection (M-C-2) from northern Utah, is similar to Races 5 and 7. These 3 races are clearly differentiated, however, by the reaction of *Bromus carinatus* and *B. catharticus.* Race 5 infects both species; Race 7 infects only *B. catharticus;* and Race 9 infects only *B. carinatus.*

Race 10 is represented by collection M-Z-1 from *Bromus ciliatus* L., collected near Bozeman, Montana. Only *B. catharticus* and *Elymus canadensis* are susceptible to this race. It probably is related to Race 7 but differs from this race by the resistance of *E. glaucus, E. sibiricus* or *Hordeum nodosum.*

Race 11 comprises several collections obtained in California from *Bromus rubens* L. The reactions of 3 of these collections (M-S, M-S-1 and M-S-2) are in Table 1. This race produces latent or normal infection on *B. japonicus, Elymus canadensis,* and *E. sibiricus.* In California and Nevada, *B. rubens* is very abundant over extensive areas and frequently is heavily smutted. Therefore Race 11 probably is one of the most widespread of all the races of head smut.

Race 12 includes 14 collections, the majority of which were on mountain brome (*Bromus marginatus* Nees.). It is one of 4 closely related races attacking this valuable forage grass. These 4 races (5, 7, 9, and 12) can be separated on the basis of their reaction on 3 of the host differentials: *Bromus carinatus, B. catharticus,* and *Hordeum nodosum.* Race 5 produces smut on all 3 species; Race 7 does not smut *B. carinatus;* Race 9 does not smut *B. catharticus;* and Race 12 does not smut *H. nodosum.* Race 12 is represented in Table 1 by the reaction of collections M-C-5 from *B. marginatus* collected at Pullman, Washington; M-C-8 from *B. polyanthus* Scribn. collected at Aberdeen, Idaho; and M-Z from *B. ciliatus* L. collected at Pullman, Washington.

Recently, heavy infestations of head smut have been reported on Bromar (4), a variety of mountain bromegrass previously resistant to all known races of head

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[2] Alaska, California, Colorado, Hawaii, Idaho, Kansas, Montana, Nebraska, Nevada, North Carolina, North Dakota, Oregon, South Dakota, Texas, Utah, Washington, Wisconsin, and Wyoming in the United States; Canada and Australia.

[3] Letter-number combinations in parenthesis refer to accession numbers; P numbers are those of the Pullman unit of the Soil Conservation Service, Division of Nurseries, F numbers are those of G. W. Fischer.

TABLE 1.—*Reaction of 11 differential species of grasses to collections of Ustilago bullata representing new races 9, 10, 11, and 12*

Differential Species	Collection Symbol, Year of Inoculation, and Per Cent Smutted Heads													
	M-C-2	M-Z-1			M-S		M-S-1	M-S-2	M-C-5		M-C-8		M-Z	
	1941	1943	1947	1948	1939	1940	1947	1948	1943	1948	1943	1948	1947	1948
B. carinatus	100	0	0	0	0	0	0	0	100	78	90	100	100	27
B. catharticus	0	24	52	19	0	0	0	0	77	67	13	19	86	43
B. hordeaceus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. japonicus	0	0	0	0	100[b]	100[b]	100[b]	14	0	0	0	0	0	0
B. mollis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. secalinus	0	0	–	0	0	0	0	0	0	0	0	0	0	0
B. tectorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. canadensis	100	–[a]	77	28	100	100	100	92	100	70	82	80	13	33
E. glaucus	0	0	0	0	30	0	0	0	0	0	0	0	0	0
E. sibiricus	0	0	0	0	–	60	13[b]	31	0	8[b]	0	2[b]	4	16
H. nodosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Race affiliation	9	10	10	10	11	11	11	11	12	12	12	12	12	12

[a] Not inoculated (indicated by —).
[b] Latent Sori.

smut. From the results of preliminary inoculations of the differential species of grasses, this strain appears to be a variant of Race 5 or Race 12. However, further inoculation studies are necessary to determine the exact relationship of this strain to established races.

VARIATION IN THE REACTION OF GRASS DIFFERENTIALS TO SMUT RACES.—Year-to-year variation in the reactions of chlamydospore collections of smuts to differential host varieties has been recognized by many investigators (1, 3, 5, 6, 7) as a problem in race identification. Variability encountered in the identification of physiologic races of head smut of grasses has been expressed in 2 ways: the smutting of certain differentials in one year and not in another, and the production of latent sori one year and normal sori in another.

This behavior could be due to several factors, including heterogeneity of inoculum. Therefore a study was made in which this factor was reduced to a minimum by using as inoculum paired monosporidial lines. Using such inoculum, the differential species were inoculated with 39 collections representing all the races of head smut. The results of 2 years' inoculations, 1947 and 1948, using the same paired lines, are too long to reproduce here. Instead, those of certain representative collections are in Table 2.

Table 2 shows that, in general, the results of the 1947 inoculations are in close agreement with those of 1948. In some cases, however, smut was produced on a grass differential in one year and not in the other. In such cases the percentage of infection usually was low and coupled with a latent type of sporulation, so that if no smut appeared in a given year it is possible that the row might have escaped infection. In most instances smut percentages were considerably lower

TABLE 2. *Variation in the reaction of the differential species of grasses to identical inoculum of Ustilago bullata in different years*

Differential Species	Collection Symbol, Year of Inoculation, and Per Cent of Smutted Heads													
	M-A-5[a]		N-T		M-I-3		M-I-4		N-O		N-V		M-E	
	1947	1948	1947	1948	1947	1948	1947	1948	1947	1948	1947	1948	1947	1948
B. carinatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. catharticus	0	0	0	0	97	94	97	90	0	0	0	0	0	0
B. hordeaceus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. japonicus	84[b]	43[b]	30[b]	21[b]	0	0	0	0	0	0	0	0	92	90
B. mollis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. secalinus	–[c]	0	–	0	–	0	–	0	–	0	–	0	–	0
B. tectorum	98	100	85	100	0	0	0	0	0	0	0	0	0	0
E. canadensis	0	0	0	0	91	92	100	95	81	24	68	12	0	0
E. glaucus	6[b]	7[b]	10[b]	40	0	6[b]	0	19	100	54	10	0	0	0
E. sibiricus	0	0	5[b]	0	0	23[b]	25	16[b]	98	80	36	19	0	0
H. nodosum	0	0	0	10[b]	0	7[b]	0	0	50	10	97	80	0	0
Race affiliation	2		2		7		7		1		1		6	

[a] Pertinent data on collections are: M-A-5 collected on *Bromus tectorum* at Elco, B. C.; N-T on *Agropyron semicostatum* at Lincoln, Nebr.; M-I-3 on *B. catharticus* at Chapel Hill, N. C.; M-I-4 on *B. catharticus* at Gelgandra, New South Wales, Australia; N-O on *A. smithii* at Pullman, Wash.; N-V on *Elymus macounii* at Chewelah, Wash.; and M-E on *B. squarrosus* at Pullman, Wash.
[b] Latent sori.
[c] Not inoculated.

TABLE 3.—*Comparative results of different kinds of inoculum of the same races in Ustilago bullata*

Differential Species	Race 2 (M-A-1) Type of inoculum[a]			Race 7 (M-I) Type of inoculum			Race 12 (M-V) Type of inoculum		
	A	B	C	A	B	C	A	B	C
B. carinatus	0[b]	0	0	0	0	0	9	84	90
B. catharticus	0	0	0	14	100	90	34	90	60
B. hordeaceus	0	0	0	0	0	0	0	0	0
B. japonicus	27[c]	91[c]	14[c]	0	0	0	0	0	0
B. mollis	0	0	0	0	0	0	0	0	0
B. secalinus	0	0	0	0	0	0	0	0	0
B. tectorum	88	100	41	0	0	0	0	0	0
E. canadensis	0	0	0	30	100	95	41	36	39
E. glaucus	6[c]	9[c]	2[c]	7[c]	19[c]	1[c]	0	0	0
E. sibiricus	3[c]	28[c]	0	6	7	32	21	20	30
H. nodosum	2[c]	5[c]	0	0	2[c]	0	0	0	0

[a] A Inoculum consisted of smut spores from field collections.
B Inoculum consisted of smut spores from host differentials that had been inoculated with paired monosporidial lines.
C Inoculum consisted of paired monosporidial lines from a single spore.
[b] Per cent smutted heads.
[c] Latent sori.

in 1948 than in 1947. Type of sporulation also varied from year to year in some instances. This was expressed by development of latent sori on a given differential species when inoculated with paired cultures one year and normal sori on the same differential another year, when inoculated with the same cultures. Much of this year-to-year variation occurred on certain differential hosts and when certain races were used. For example, *Elymus glaucus*, *E. sibiricus*, and *Hordeum nodosum* reacted in a uniform manner to most races with respect to infection and the type of sorus produced. However, when certain collections of Races 2, 7 (Table 2), or 12 (Table 1) were used the reaction varied from year to year, and collection to collection. Thus, the results of the 2-year test show clearly that collections of head smut may react differently even when comparable inoculum is used.

However, it was found that nearly all of the 39 collections tested for pathogenicity using paired monosporidial lines could readily be placed in the same races differentiated by using smut spore inoculum. In addition, when spores and paired monosporidial lines were compared in a parallel series of inoculations in the same year, the results were comparable, although some variations occurred. Results of such a paired series of inoculations are in Table 3.

Results suggest that smut spore inoculum is reasonably homogenous and reliable for purposes of race identification of field collections of head smut.

DISCUSSION.—Year-to-year variation in reaction of the differential host species to collections of head smut when identical inoculum is used indicates that apparent variation in pathogenicity is not due to impurity of inoculum. It is possible that such variation may be due to heterozygosity of the grass differentials or to varying environmental factors affecting host-pathogen

TABLE 4.—*Differentiation of 12 pathogenic races of Ustilago bullata by their reaction on 11 species of grasses*

Differential species	Acc. No.	Races											
		1	2	3	4	5	6	7	8	9	10	11	12
Bromus carinatus	P-2725	R[a]	R	R	R	S	R	*R*	R	S	R	R	S
Bromus catharticus	F-161	R	R	R	R	S	R	S	R	*R*	S	R	S
Bromus hordeaceus	F-155	R	R	R	s	R	R	R	S	R	R	R	R
Bromus japonicus	F-154	R	s[b]	R	R/S/s	R	S	R	S	R	R	*s*	R
Bromus mollis	F-194	R	R	R	*S*	R	*R*	R	R	R	R	R	R
Bromus secalinus	F-329	R	R	R	R	R	R	R	*S*	R	R	R	R
Bromus tectorum	F-165	R	*S*	R	R	R	R	R	R	R	R	R	R
Elymus canadensis	P-2389	S	R/s[c]	S	S	S	R	S	s	S	S	*S*	*S*
Elymus glaucus	P-1851	S	R/S/s	*R*	R	R	R	R/S/s	R	R	*R*	R/S	R
Elymus sibiricus	P-225	*S*	R/S	*R*	R	R/s	R	R/S/s	R	R/s	*R*	S/s	R/S/s
Hordeum nodosum	P-2723	S	R/s	S	R	*S*	R	R/S/s	R	R	*R*	R	*R*

[a] R = immune or highly resistant.
S = susceptible, with production of normal sori.
S = italicized symbols signify differential reactions.
[b] s = susceptible, but with the production of latent sori.
[c] R/S/s = reaction variable.

compatibility. When spore inoculum is used, there is additional possible variation due to heterozygosity of the chlamydospores, or to slight differences in genotypes of different collections. Under such circumstances a single race may include many biotypes differing slightly in pathogenicity.

With these considerations in mind and to facilitate the identification of races of head smut, the limits of differentiation of the 8 physiologic races given in the previous paper (2) have been broadened somewhat in consideration of variation in reactions of races to the differential species (Table 4). In addition, the differentiation of the new races 9 to 12 is included.

It appears from the results obtained in this investigation that in *Ustilago bullata* the use of paired monosporidial lines is no more effective in the long run than mass smut spore collections in establishing physiologic races. However, the use of monosporidial lines has certain advantages over spores in host specialization studies. For one thing it is possible to attain much more quickly the same homogeneity of inoculum possible by the use of varietal screening. There is no evidence that, in head smut, this method eliminates any pathogenicity complexes necessary for varietal resistance tests as reported in the oat smuts (3). Another advantage of using paired monosporidial lines is that collections of smut which are too meager to test by smut spore inoculations can be increased in culture. A further advantage is that it reduces the chance for contamination by air borne smut spores, always a possibility when large numbers of smut collections are being tested simultaneously. A fourth advantage is that if the same paired monosporidial cultures are used in a series of inoculations over a period of years, variation due to environment and host can be distinguished from that due to segregation and recombination which may occur when mass smut spore inoculum is used, providing mutations or loss of pathogenicity in culture do not occur.

SUMMARY

Additional investigations on physiologic specialization in head smut of grasses (*Ustilago bullata*) have resulted in the differentiation of 4 additional races (9, 10, 11, and 12) and in the reduction of the number of differential grass species from 14 to 11.

Additional studies using both paired monosporidial lines and spores as inoculum have shown that variation in the response of differential grass species to smut collections exists, regardless of purity of inoculum. As a result, the descriptions of the races have been modified to allow for such variability.

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ORGANIZATION OF CROWN-GALL TUMOR CELLS IN THE PRESENCE OF A FORMATIVE STIMULUS [1]

Armin C. Braun

Recent studies[2,3] have demonstrated that organization and ultimate recovery of bacteria-free crown-gall tumor cells may be obtained in instances where the tumor tissue was originally derived from cells possessing highly developed potentialities for organization. In such instances the pluripotent tumor cells retain indefinitely in culture a well developed capacity for organizing small abnormal leaves and buds. By forcing the rapid growth of such tumor buds complete recovery of the affected cells was accomplished. In tumor tissues of this type the inherent potentialities of the cells for organization are so great that they retain, despite their transformation, a strong organizational capacity. This contrasts sharply with results obtained when plant cells possessing a low competency for organization are transformed by the bacteria. In the latter instance the tumor cells are characterized both in the host and in culture by their very limited powers of differentiation and, more strikingly, by their apparent inability to organize. It was of interest to learn next, therefore, whether crown-gall tumor cells of the mostly undifferentiated and completely unorganized type could be made to organize structures if such tumor tissues were subjected to a sufficiently profound externally applied formative stimulus.

The transformation of normal plant cells to crown-gall tumor cells in a number of plant species including the periwinkle (*Vinca rosea* L.) appears to take place progressively, leading in a 3–4 day period to a completely autonomous, rapidly growing type of cell.[4] Cells of the periwinkle altered in shorter periods (34–36 hours), on the other hand, commonly develop slowly in the host and in culture. One such clone, described briefly elsewhere[4] and used in the experiments reported here, has now been maintained in culture for somewhat more than 6 years. During this period the tissues comprising this clone have developed very slowly when compared under similar conditions with cells transformed by the bacteria in 3 or 4 days (Fig. 1, A and 1, B). While the latter were glistening white in color and loose in texture, the cells transformed in 34 hours developed as firm masses of tissue that were white or light brown. Greenish patches frequently developed on their surfaces. The 2 types of tumors were transplantable and, when sterile fragments of the tissues were implanted into healthy periwinkle plants, they developed into neoplastic overgrowths. During the 6-year period that these tissues have been cultivated on White's medium they have never been found to organize either roots or shoots. The pronounced difference in the growth habits of the 2 types of tumor tissues in the host and in culture gives the unmistakable impression that the alteration brought about by the bacteria in 34 hours represents a lower grade of neoplastic transformation than that found in cells altered in a 3 or 4-day period.

Although the influence of auxins of various types on the development in culture of rapidly growing completely altered crown-gall tumor cells of the periwinkle has been studied,[5] there is no record indicating that these substances encouraged the initiation of roots or shoots in the tumor tissues. Since, however, cells altered by the bacteria in 34 hours in the periwinkle gave every indication of representing an earlier stage in the transformation process, it was of interest to learn whether these slowly growing tumor tissues were capable of responding to certain morphogenetic stimuli that are effective against normal tissue but are, as far as is known, ineffective against fully altered tumor cells.

Normal periwinkle stem cuttings root easily in a suitable environment. It seemed reasonable to suppose, therefore, that potentialities for root initiation were well developed in cells of that species. To encourage any potentialities for root initiation that might have been retained by the tumor cells, fragments of the tumor tissues were cultivated in the presence of α-naphthalene acetic acid, a highly active root-inducing substance which was incorporated into White's medium in concentrations of 1.0 mg., 0.1 mg., and 0.01 mg. per liter. Small fragments of tumor tissue, about 2 mm. square and 1 mm. thick, of the type transformed by the bacteria in 34 hours were planted on White's medium containing the 3 different concentrations of growth substance. As controls, similar fragments of tumor tissue were grown on White's medium without added growth substance. The experiment was performed on 2 different occasions.

Results of these experiments showed that the tumor tissues developed slowly and in a typical unorganized manner in the 3 concentrations of growth substance as well as in the control flasks. The presence of α-naphthalene acetic acid in the medium did not appear to stimulate the rate of growth of the tumor tissues. About 1 month after the tissues had been planted on the growth-substance-containing media, the initiation of roots was observed in some of the fragments. In

[1] Accepted for publication January 2, 1953.

[2] Braun, Armin C. 1951. Recovery of crown-gall tumor cells. Cancer Res. 11: 839-844.

[3] Braun, Armin C. 1953. Bacterial and host factors concerned in determining tumor morphology in crown gall. Bot. Gaz. In press.

[4] Braun, Armin C. 1951. Cellular autonomy in crown gall. Phytopathology 41: 963-966.

[5] deRopp, R. S. 1947. The response of normal plant tissues and of crown-gall tumor tissues to synthetic growth hormones. Am. J. Bot. 34: 53-62.

the first experiment of 53 tumor fragments planted on the growth substance, 7 (about 13 per cent) had developed 1 or more roots at the end of an 8-week period (Fig. 1, C). Of the 40 control cultures used, none had produced a root at the time the experiment was terminated.

In the second experiment, of the 60 tumor tissue fragments tested in the presence of α-naphthalene acetic acid, 11 (about 18 per cent) produced from 1–23 macroscopically visible roots at the end of an 8-week period. Roots were not found to be present on any of the 60 control cultures. Of the 3 concentrations of α-naphthalene acetic acid used, all seemed to be about equally effective in inducing the initiation of roots in the tumor tissues.

Experiments similar to those reported above were carried out with the use of completely altered, rapidly growing periwinkle tumor tissues. Roots were not observed to develop from any of the tumor fragments cultivated in the presence of α-naphthalene acetic acid or in the control cultures.

Results of this study indicate that it is possible to cause crown-gall tumor cells possessing a low grade of neoplastic transformation to organize roots in the presence of a root-inducing substance. They suggest further that an organization and ultimate recovery of completely altered tumor cells of the type that possess a very low competency for organization might possibly be accomplished if a sufficiently powerful morphogenetic stimulus were found and applied to those cells.

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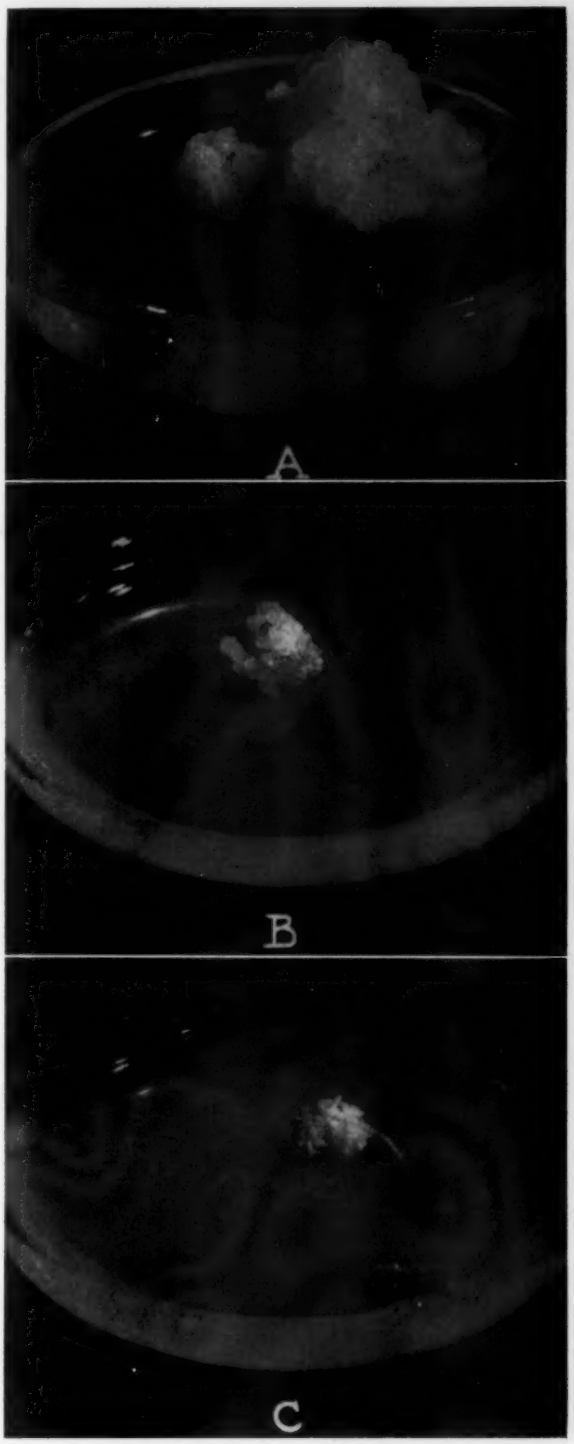

Fig. 1. A) growth, on White's medium, of periwinkle crown-gall tissue originally derived from healthy cells transformed by bacteria to tumor cells during a 4-day period. B) growth, on White's medium, of crown-gall tumor cells originally derived from healthy cells transformed by the bacteria to tumor cells during a 34-hour period. C) crown-gall tumor cells similar to those shown in B but grown on White's medium containing 0.1 mg./liter of α-naphthalene acetic acid. Note development of well organized roots of various lengths.

SUPPRESSING EFFECT OF RADIOACTIVE PHOSPHORUS ON SYMPTOMS AND VIRUS CONTENT OF MOSAIC TOBACCO PLANTS [1]

David E. Schlegel, A. H. Gold, and T. E. Rawlins

Although tobacco mosaic virus (TMV) infected tobacco plants, grown on nutrient solutions containing radioactive phosphorus (P^{32}), have been shown to take up the phosphorus and incorporate it into the virus (6), no report has been found on the effect of radioactivity on plant virus multiplication, except the earlier abstract of the present work (4). Preliminary attempts to label TMV massively with C^{14} (Delwiche and Gold, unpublished) in concentrations greater than 10 millicuries (mc.) per gm. fresh weight of tissue resulted in a significant decrease in the amount of virus produced.

In the experiments to be described, high levels of P^{32} activity were used, since these conditions would be expected to be most unfavorable for virus multiplication.

METHODS.—Healthy and TMV infected tobacco seedlings were grown in Hoagland's (1) nutrient solution containing varying amounts of P^{32}. The seedlings were transferred from soil to complete nutrient solution about 1 week before the addition of P^{32}. During this period the plants recovered from the initial transfer shock and grew vigorously. Fresh nutrient solutions which contained only half the normal amount of phosphorus were prepared and poured into 12 pyrex containers. Each container held 1,800 ml. of nutrient solution. The radioactive phosphorus (K_2HPO_4 — KH_2PO_4 containing .025 mg. P per mc.) was added to these in such a manner that there were 2 dishes containing each of the following concentrations of the isotope: 0, 0.44, 1.33, 3.5, 6.2, and 12.0 mc. Three seedlings were placed in holes previously drilled through the pyrex lid of each dish. The plants in 1 series of concentrations were immediately inoculated with TMV by rubbing each of the older leaves with a pad of cotton saturated with a suspension of infective sap and carborundum. The other series was left noninoculated. This arrangement provided both noninfected and TMV inoculated plants at various levels of radiation from 0–12 mc. per 1,800 ml. of culture solution. At the end of 10 days the plants were harvested, weighed, and frozen in preparation for the virus assay. Each lot of 3 plants was broken down into 2 portions, one consisting of the upper, young leaves which had grown out after inoculation, and the other, the older, lower leaves which, in 1 series, had been inoculated.

Six samples for virus assay were obtained from the older leaves of each lot by punching discs from frozen leaves with a cork borer. Since the upper leaves were too small to supply discs, the entire leaf was utilized in the virus assay.

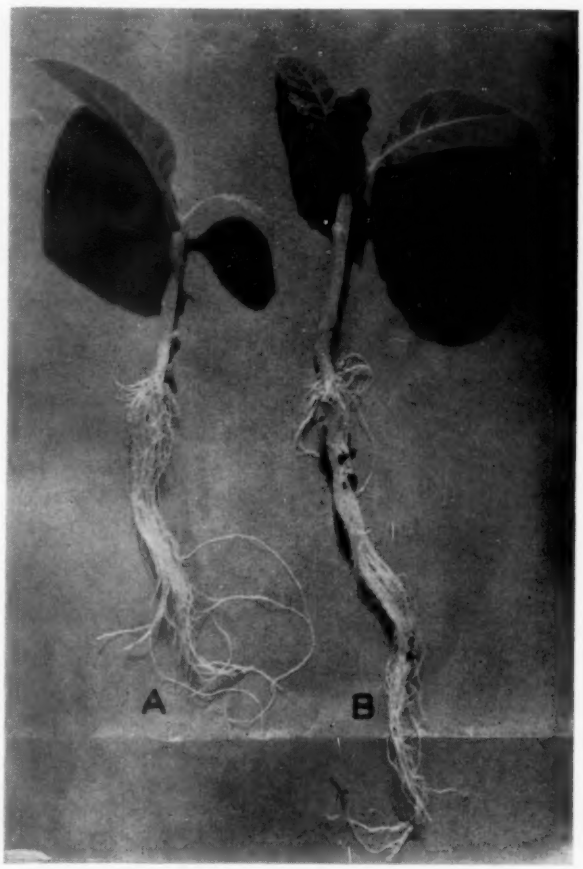

FIG. 1. The inhibiting effect of high concentrations of P^{32} on root tips and apical meristem of healthy tobacco seedlings. A) Grown on nutrient solution containing 12 mc. P^{32}. B) Grown on non-radioactive nutrient solution.

The virus assay method (5) consisted of homogenization and dilution of the frozen, weighed samples, followed by heating to 60° C. for 15 minutes to coagulate the host proteins. The coagulum was removed by a centrifugation at 8,000 × gravity, and the virus was sedimented from the supernatant liquid by a centrifugation at 75,000 × gravity. The virus pellet obtained in the latter centrifugation was dissolved in distilled water and subjected to another centrifugation at 8,000 × gravity. The relative virus content of the supernatant was estimated by measuring its absorption at 260 mμ and comparing this value with the curve of Takahashi (8). The virus concentration was expressed as mg. of purified virus extracted per gm. of fresh tissue.

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Radioactivity determinations were then made on samples of the homogenate. Since the plants contained high levels of radioactive phosphorus, only small amounts of homogenate were needed. A nitric acid hydrolysis was found adequate to solubilize the homogenate. The hydrolysates were neutralized, diluted, evaporated to dryness, and the activity determined.

RESULTS.—Several abnormalities in the growth of the plants at high radiation levels were observed. Roots showed a decidedly abnormal habit of growth characterized by an inhibition of terminal development and the appearance of swellings along the roots. (Fig. 1, 2). The aerial parts of the plant also showed radiation damage characterized by a marked suppression of growth of the apical meristem (Fig. 1) and a decided tendency for formed young leaves to become more or less oblanceolate in shape, whereas the normal young leaves became more typically ovate (Fig. 3). The older leaves showed none of these characteristics and appeared to increase in size in a normal manner.

These qualitative observations on both roots and leaves are in accord with the view that cell division and the formation of new nuclear material are arrested by certain levels of radiation, whereas at these same levels, cell enlargement may actually increase. This view is borne out by observations of Quastler and Baer (3) who studied the effect of X-ray radiation on the initiation and completion of growth in mung beans, and found cell division much more radiosensitive than cell enlargement. Mackie, Blume and Hagen (2) reported similar results in barley grown in P^{32}.

Although the younger leaves of plants grown on the higher concentrations of P^{32} were somewhat deformed, typical mosaic symptoms were markedly reduced (Fig. 3). Mosaic symptoms were almost entirely lacking on young leaves from plants grown on the highest levels of radioactivity, and as the radioactivity of the nutrient solution decreased, the mosaic symptoms became more pronounced.

Inhibition of virus multiplication by P^{32} was indicated by the results of the virus assays made on young leaves which grew out after inoculation (Fig. 4). Over a relatively short range of P^{32} concentration (100-300 counts per second per mg. of tissue) the virus yield was roughly inversely proportional to the radioactivity in the tissue; increasing the activity to more than 300 counts per second per mg. of tissue did not significantly reduce the virus yield. The relatively small effect of increasing the P^{32} content above 300 counts may be due to there being some virus multiplication in these leaves before the P^{32} increased sufficiently to prevent virus formation.

The older inoculated leaves did not, however, show a reduction in virus content with increasing radioactivity. Some differences were noted but were not considered significant. Comparisons of the specific radioactivity of the younger and older leaves showed that the younger leaves had specific activities 2–2.5 times as great as the older leaves.

DISCUSSION.—The results of this experiment indicate that the presence of P^{32}, an element which is present in the structure of the virus particle, can cause a reduction in the virus yield of mosaic tobacco plants. No information is available regarding the effect of other radioactive elements which are not a part of the virus particle.

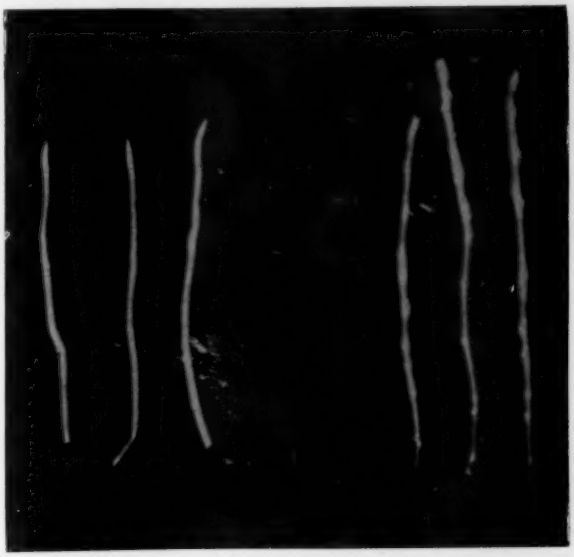

FIG. 2. Effect of high levels of radiation on the roots. The roots on the left are normal; those on the right are from plants grown on the highest level of radioactivity, and show distinct malformations.

The failure of P^{32} to reduce the virus yield in the older leaves may be due to several factors. Since the older leaves were inoculated with a suspension of concentrated virus, a large number of infections on each

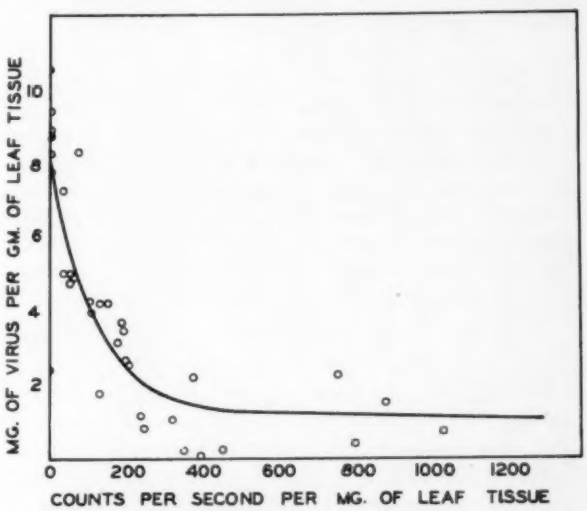

FIG. 4. Effect of different concentrations of P^{32} on quantity of virus recovered from young leaves. The virus yield is plotted against radioactivity of the leaf tissue from which the virus was obtained. The curve shows that the virus yield decreases as the radioactivity in the leaf tissue increases to around 300 counts. Each point (o) represents the assay of 1 leaf.

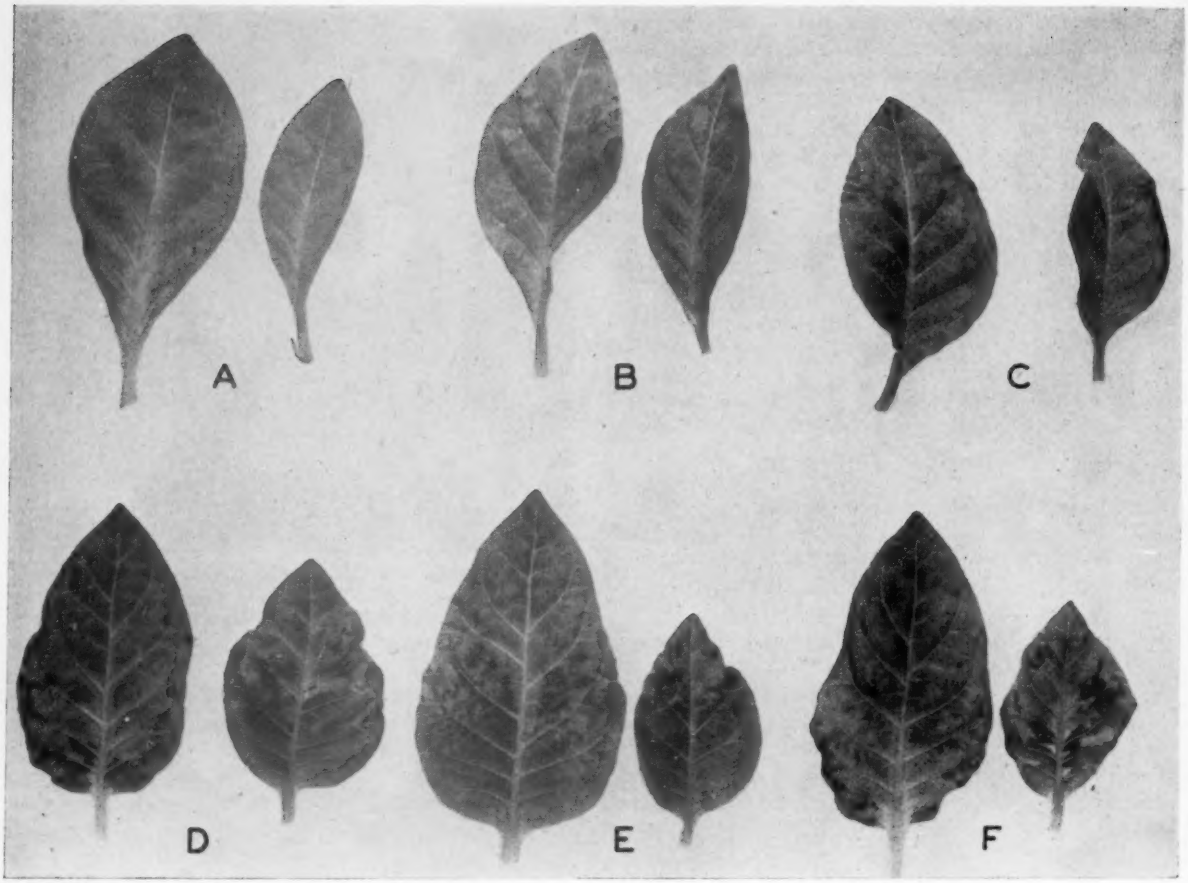

FIG. 3. The effect of different concentrations of radioactive phosphorus on the expression of tobacco mosaic symptoms in comparable young tobacco leaves. All the leaves shown are young, infected leaves, and each level of activity is represented by 2 leaves, a very small leaf and a somewhat larger leaf. A = 12, B = 6.2, C = 3.5, D = 1.33, E = 0.44 F = 0 mc. of P^{32} in the 1,800 ml. of nutrient solution in which the plants were grown. Note the suppression of symptoms and oblanceolate shape of leaves grown on the higher levels of activity (A, B, and C).

old leaf should have resulted. Tobacco mosaic virus has been shown by Steere (7) and others to reach its maximum concentration very quickly after inoculation and, therefore, it is possible that virus multiplication in inoculated leaves may have been essentially completed before sufficient P^{32} had accumulated in these leaves to cause a reduction in virus yield. In addition, the older leaves contained only about half as much P^{32} per mg. of fresh tissue as the younger leaves.

It appears that the threshold for virus inhibition was roughly around 100 counts per second per mg. of tissue and that for observed plant injury was around 200 counts; this difference may be too small to permit the application of P^{32} in chemotherapy of virus diseases. On the other hand, it is possible that in certain other virus diseases the differential susceptibility of host and virus to P^{32} may be greater, and some applications may be feasible.

Some of the factors which may be involved in the inhibition of mosaic symptom expression in leaves containing high levels of P^{32} are the decreased virus content and the effect of P^{32} on host metabolism. Little evidence is available regarding the relative importance of these factors in the inhibition of symptoms.

SUMMARY

Radiophosphorus (P^{32}) in young mosaic tobacco leaves inhibited virus formation and mosaic symptom expression. P^{32} activity of around 100 counts per second per mg. fresh weight of leaf tissue decreased virus formation to approximately half that in control leaves; 200 counts per second decreased it to around ¼ that in controls.

Levels of P^{32} of 200 or more counts visibly inhibited the terminal growth of stems and roots, but the enlargement of formed leaves was less susceptible to inhibition by P^{32}.

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BRASSICA NIGRA VIRUS TRANSMISSION SOME VECTOR-VIRUS-HOST PLANT RELATIONSHIPS[1]

Edward S. Sylvester[2]

The *Brassica nigra* virus described in 1949 (22), was considered to be distinct from the many other reported crucifer viruses because of symptom tolerance to high temperatures and the failure to transfer the virus to tobacco. Some aspects of its transmission by aphids have been reported (20, 21) and in general the vector-virus relationships are of a nonpersistent type (25). Among phases of virus transmission by insects that have lacked sufficient investigation are those concerned with the influence of various conditions affecting plants used in transmission trials. The following work is primarily concerned with results of experiments designed to determine the influence on transmission of such factors as site of inoculation, effect of darkness and age on susceptibility to inoculation, effects of age on active virus concentration, and variation in transmission due to differences among virus sources.

Materials and Methods.—The virus was originally obtained from Dr. W. N. Takahashi, and since has been maintained in smooth-leaf mustard plants, *Brassica juncea* Czern. et Cross. Test plants were mustard seedlings unless otherwise noted. The vector used was the green peach aphid, *Myzus persicae* (Sulz.), and noninfective colonies were maintained on healthy mustard plants. Insect transfers were made with a camel's-hair brush and acquisition feedings, unless otherwise noted, were watched and timed with a stop-watch. The plants were fumigated after test feedings before being placed in the greenhouse for incubation. The greenhouses were routinely sprayed to prevent aphid build-up. More detailed descriptions of methods are given in connection with specific experiments.

Results.—*Variation due to aphid-host plant interaction.*—Variations between replications of transmission experiments presumably are due to individual differences in aphids, test plants, and virus sources. Virus source can be limited to 1 plant, or a single leaf, which should reduce this variable to the practical minimum. Aphids can be limited to progeny of 1 individual which, because of parthenogenic reproduction, should approach a homozygous condition. Test plants are usually limited to samples from 1 seed lot, and presumably would have individual variations of a greater magnitude than the aphids. The following experiments are concerned with evaluation of the above mentioned variables.

One-hundred green peach aphid apterae selected at random from a single population were divided into 10 equal lots. Following a preliminary fasting period with a mean (range) value of 85 (60–116) minutes, the insects were fed singly on the upper surface of 1 leaf of an infected plant. Feeding was restricted to 1 15-second penetration, and the aphid was then transferred immediately to a test plant. Test plants were mustard seedlings in the cotyledon stage which had been selected from a uniform population.

The first 10 aphids used were designated sample A, the next 10 sample B, etc., until 10 samples were completed. This procedure introduced a bias because of the increasing fasting time as the samples were being completed, but evidently the fasting range used did not influence the results. The mean number of plants infected out of 10 possible was 5.9, with a range of 3–7. Results were uniform, and support the hypothesis that some degree of effective standardization in transmission experiments can be obtained through the use of single virus sources, single colonies of insects, and uniform test plant populations.

Effect of site of inoculation on transmission.—Frequently it has been observed during experimental work that individual insects feed on different areas of test plant seedlings. It is possible that various tissue regions differ in their susceptibility and consequently the site of inoculation could influence experimental results.

[1] Accepted for publication January 12, 1953.

[2] Assistant Professor of Entomology and Assistant Entomologist in the Experiment Station, University of California, Berkeley, California.

TABLE 1.—*Results of tabulations to determine aphid feeding preference and susceptibility of various plant areas in connection with transmission of the Brassica nigra virus by green peach aphids. The plants inoculated were mustard seedlings, and the test feedings were approximately* 15 *seconds*

Area of feeding	Number of aphids recorded feeding	Number of plants inoculated	Per cent of successful inoculations
CU[a]	176	9	5.1
CL	714	37	5.1
CP	381	28	7.3
S	163	11	6.7
LP	60	9	15.0
LL	87	6	6.8
LU	26	2	7.6
J	7	0	0.0

[a] The meanings of the various symbols are: CU—upper surface of cotyledon, CL—lower surface of cotyledon, CP—petiole of cotyledon, S—stem, LP—petiole of leaf, LL—lower surface of first leaf emerging, LU—upper surface of first emerging leaf, and J—junction of the stem, cotyledon, and leaf petioles, respectively.

During a series of transmission trials a record was made of the sites of inoculation. The test plants were in the cotyledon stage with the first true leaf beginning to emerge. Eight general feeding areas were arbitrarily designated and records were kept on the frequency with which insects fed on the areas and the results of the inoculations. The areas were: cotyledon, upper surface (CU); cotyledon, lower surface (CL); cotyledon, petiole (CP); stem (S); leaf petiole (LP); leaf , lower surface (LL); leaf, upper surface (LU); and junction of the stem, cotyledon, and leaf petioles (J). The timed, watched test feedings were approximately 15 seconds, and the aphids were initially placed on the cotyledon upper surface. In approximate percentages, 11 per cent of the aphids fed on the cotyledon upper surface (Table 1), while 44 per cent crawled to the cotyledon lower surface to feed. Of those which continued the initial wandering beyond the cotyledon surfaces, some 24 per cent fed on the cotyledon petiole, or the junction of the stem, cotyledon, and leaf petioles. Four per cent of observed individuals fed on leaf petioles, 5 per cent on the lower and 0.2 per cent on the upper surfaces of the newly emerging leaf.

Little difference in susceptibility existed among the various designated plant regions. The petiole of the youngest leaf was apparently a little more susceptible. However, it is unlikely that experiments would be materially affected by this difference, because of the low probability of this area being fed on, and theoretically the contacts would be distributed at random.

Effect of darkness on plant susceptibility.—Several reports in the literature indicate that plant susceptibility to virus infection can be influenced by light (14, 15, 17, 24). In these accounts differences in susceptibility were determined by using local lesion reactions and not systemic infections.

When restricted to single penetrations, insects hypothetically introduce relatively small amounts of virus in superficial layers of plants. Consequently, the amount of infection occurring during restricted feeding could be influenced by factors affecting plant tissue susceptibility.

The inoculation threshold period is between 6 and 10 seconds, using the *Brassica nigra* virus and the green peach aphid (2). If inoculation feedings are limited to this range, vector efficiency is less than when they are longer. The lower efficiency might be due to failure of insects to penetrate to susceptible tissues, or the inoculum introduced in many cases could be too small to overcome resistance of tissues to infection. If the latter is true then any treatment which would increase tissue susceptibility might be reflected in vector efficiency increase when using inoculation feedings of near threshold value. The hypothesis was tested by the following experiments.

Test plants were preconditioned before inoculation as follows. In a uniform population 10 plants were covered with 2-in. clay pots, the holes of which had been stopped with corks. Twenty-four hours later another sample of 10 was covered in the same manner. The following morning the covered plants, along with an additional sample of 10 plants, were inoculated. The additional 10 plants had a normal period of darkness, and were labeled the *morning* inocualtion. Inoculation was done by using a 10 second feeding by an infective green peach aphid. The aphids had been previously starved for an average period of 1.4 hours, and then were given 2 acquisition feedings averaging 14.8 seconds on a virus source. In the afternoon of the same day, 10 more plants were inoculated in a like manner. These plants had had approximately 10 hours of daylight and were labeled *afternoon* inoculation. Five replications, 10 plants per treatment, were done.

Little change in suscepitbility of mustard seedlings to infection by the *B. nigra* virus was caused by any of the treatments (Table 2). Either plant susceptibility was not influenced by a period of darkness preceding inoculation, or else variations in the ability of aphids to inoculate seedlings under the experimental conditions were not due to light sensitive differences in tissue susceptibility. In spite of the negative results obtained, the method might be used in test with other

TABLE 2.—*Results*[a] *of trials to determine the influence of darkness on susceptibility of mustard seedlings to infection with the Brassica nigra virus inoculated by fasted infective single green peach aphid apterae. The test feeding was restricted to* 10 *seconds*

Preconditioning treatment of plants			
In dark (hours)		*Overnight*[b]	*Afternoon*[c]
24	48		
9	13	14	9

[a] The numbers in the columns are infected plants out of 50 inoculated using 5 replications, 10 plants per treatment.
[b] Approximately 12 hours of darkness with 3 hours of daylight.
[c] Approximately 10 hours of daylight.

viruses. However, similar trials with beet-mosaic virus produced like results; and Bradley (4), using local lesion reactions in connection with both aphid and mechanical inoculation trials, reported that susceptibility of tobacco to infection by a strain of Henbane mosaic virus, could be increased by a preliminary period of darkness when using mechanical inoculation but not when using aphids.

Effect of age on plant susceptibility.—Age of test plants is another variable which might influence virus transmission experiments. Normally within replications age is constant, but between series of replications accumulation of age often occurs. Apparently this point seldom has been investigated in connection with insect transmission trials (24). In the following experiments mustard plants of different ages were inoculated in replicated trials with the *B. nigra* virus using single green peach aphids.

On 5 consecutive days in each week for 5 weeks, 3 sets of 15 mustard seedlings were transplanted into 3-in. clay pots. The plants were 5 days old when transplanted. Three weeks after transplanting the plants were given a small amount of commercial fertilizer. The plants were arranged in 5 blocks so that 2 days before the first block was inoculated there was a randomizing block design with a single restraint, consisting of 3 duplications of 5 ages replicated 5 times. The age within block was duplicated 3 times to permit the use of 3 virus sources. The plants were inoculated 2 days after the last series was transplanted by single infective green peach aphids. The aphids were given a mean preliminary fasting of 1.8 hours and then a 15 second acquisition feeding. The test feeding was approximately 1 hour and the insects were placed on the cotyledons (1-week old plants) or the youngest expanded leaf. At the time of the inoculation the number of plants per treatment was reduced from 15 to 10 and the remaining plants were cut off at the ground line, then weighed and outlined to obtain a measurement of difference in size (Fig. 1). The virus source plants had been inoculated 3 weeks prior to use and were grown in a single pot. Three out of 5 possible were selected for uniformity in size and appearance, and designated as A, B, and C. The acquisition feeding was restricted to the first leaf showing on each of the 3 plants. On virus source A the veinclearing was confined to approximately the basal ½ of the leaf, on B it was on the basal ⅛, while on C the entire leaf showed veinclearing. The inoculations were made on 5 consecutive days and the youngest age inoculated was 1 week, while the oldest age was 5 weeks. The same insect colony was used in replications I, III, IV, and V, while a different colony was used in replication II. Replications I, IV, and V were done in the morning, while I and III were done in the afternoon.

All ages were approximately equally susceptible. The variation between replications was large, the number of plants infected in replication II (11), was noticeably less than that in the others. This may have been due to the shifting to another colony of insects. The plants infected in replications IV and V (27 and 27) were less than those infected in replications I and III (51 and 58). Virus source A and B were comparable (51 and 53 infections, respectively), while there was a strong tendency for virus source C to be superior (70 infections).

FIG. 1. Graf-illustration chart showing the average differences in weight and size of the 5 ages of mustard plants used in trials to determine the influence of age on susceptibility. Note the nutritional stunting during the 3rd week of growth and subsequent response to fertilizer in the 4th week.

As can be seen from the figure, plant growth was noticeably less during the third week, but by the end of the fourth week good response had been obtained from fertilizer. Little difference existed in the mean incubation periods of the various ages, the range being from 5–11 days. The average incubation periods were: 1 week, 5.78 days; 2 weeks, 6.47 days; 3 weeks, 7.89 days; 4 weeks, 6.93 days; and 5 weeks, 7.30 days. If any conclusion is justified, it would be that the incubation period is correlated with rapidity of growth.

Effect of virus source on transmission.—Variation in experimental results attributable to differences in virus source plants has received little investigational attention in vector-virus work. In one of the original experiments on the influence of age on susceptibility of test plants, an unrestrained randomized block design was used, the infective insects being obtained by using a 5 minute access period on 3 virus source plants. When the results were tabulated there were indications that the youngest age inoculated (2 weeks) was the most susceptible to infection (32 per cent infection), and that 4-week old plants were somewhat resistant (2 per cent infection), with the remaining ages, 3, 5, 6, and 7 weeks old varying from 8–14 per cent infection. The percentages are based on samples of 50. However, when tabulations were made on the basis of virus source, it became apparent that large differences existed among the 3 sources used in spite of the fact that all were subinoculations by single insects from 1 plant, were of the same age, and were selected because of uniform appearance. Based upon samples of 100 for each virus source, the percentages of infection were: virus source I, 25 per cent; source II, 13 per cent; and source III, 1 per cent. It was not certain,

TABLE 3.—*Results of trials to determine influence of age of infection on transmission of Brassica nigra virus by single fasted green peach apterae. Single leaves of 5 infected mustard plants served as virus sources. Daily virus recovery trials were made from each source to 10 healthy mustard seedlings. Recoveries were begun 8 days after inoculation when the first symptoms appeared. Acquisition feedings were approximately 15 seconds, while test feedings were approximately 1 hour*

Virus source	Age of infection in days															
no.	8[a]	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Total
I	2[b]	2	1	2	3	4	2	5	5	4	4	6	3	3	7	53
II	1	2	3	4	5	5	2	6	7	4	3	1	6	5	1	55
III	4	3	5	3	4	0	5	4	5	5	5	8	6	2	6	65
IV	1	1	3	5	4	3	5	4	8	6	7	7	6	7	2	69
V	2	1	0	2	1	2	4	6	4	4	4	4	4	5	1	44
Total	10	9	12	16	17	14	18	25	29	23	23	26	25	22	17	286

[a] The infection was 8 days old at this time, but this was the first day that symptoms appeared.
[b] Numbers in columns are number of plants infected out of 10 inoculated.

however, whether or not the high number of successful inocluations on the youngest group was due to a good virus source (40 out of the 50 inoculated were with source I), or that the superiority of source I was due to the frequency of its use on young plants.

When the data of the previously discussed experiment are considered, there would seem to be support for the conclusion that the differences just discussed were largely due to variation in virus source rather than age of the test plants. As a result of this and similar experiences, additional experiments were conducted in an attempt to evaluate the influence of virus source plants in transmission experiments.

Effect of age on active virus concentration.—The first group of trials were designed to determine differences in transmission due to virus source plants and age of infection. In addition some information was collected on the use of insects to measure relative amounts of active[3] virus in different plant areas during various stages of infection (6, 7, 9, 10, 16, 19, 24).

To establish the virus sources 10 mustard plants in the 4-leaf stage (6–9 mm. high) were selected from a population and inoculated with *B. nigra* virus using infective green peach aphids. Eight days later, when symptoms (veinclearing) first appeared, 5 plants were picked for the tests. Daily inoculations to 10 mustard seedlings were made from each plant for a total of 15 days. For virus recovery purposes aphids were given a mean (range) preliminary fasting period of 106.9 (60–155) minutes, a mean (range) acquisition feeding of 14.8 (10–19) seconds and then transferred singly to test plants for a mean (range) test feeding of 65.6 (59–133) minutes. The acquisition feeding on each source was restricted to the oldest leaf showing symptoms. On virus source plant I the fourth leaf was used, on II the sixth, on III and V the fifth, on IV the third. The cotyledons had dried on all plants except IV.

Despite the within treatment variation accentuated by the small samples (Table 3) a definite trend existed which indicated a rise and fall in active virus concentration. Approximately 15–20 days after inoculation active virus concentration appeared to reach near maximum. Thereafter active virus available to insects declined. Similar results have been reported for other viruses, e.g., alfalfa mosaic (13), as measured by local lesion methods. Consequently the possibility exists for using insects to determine peak active virus concentrations in situations where other methods are not available.

The second group of trials indicated again that insects could be used to determine change in active virus concentration in single leaves. This is difficult by other methods since these normally utilize mechanical inoculation and consequently remove irreplaceable tissue. In the following trials the path of virus infection in source plant III of the previous experiments was followed for an additional 12 days in the originally sampled leaf, and for 16 days in additional leaves.

Beginning 16 days after symptom appearance, besides the original leaf, 3 younger leaves of the same plant were sampled daily by inoculating 10 mustard seedlings from each. This was continued for 12 days. The oldest (original) leaf was dead on the 35th day, and 3 more young leaves were sampled daily. This daily sampling, now including 6 leaves, was continued for 4 days. The recoveries, using single aphids, were made in the same manner as previously described. The insects were fasted for a mean (range) period of 85.2 (61–124) minutes, given an acquisition feeding of 14.9 (10–7) seconds, and then transferred to the test plants for a mean (range) period of 61.5 (60–71) minutes.

The course of active virus concentration, as measured by aphid recovery trials, in leaf I from the time of the first symptoms until death is in Table 4. The first symptoms, 8 days following inoculation, were veinclearing, fairly discrete initially and then becoming diffuse. No mosaic mottling developed. The leaf blade length was approximately 8 mm. when symptoms were first noted and little subsequent growth occurred. By the thirty-fourth day ¾ of the leaf tissue was dry and the remaining was flaccid. One recovery trial from this

[3] The term active virus here is restricted to mean that capable of inducing infection when transmitted by insects.

TABLE 4.—*Results[a] of trials to determine amounts of active virus in a mustard plant during the course of an infection from 8–39 days old. Virus was recovered daily from 1–7 leaves by means of single green peach aphids. The aphids were fasted, given an acquisition feeding of approximately 15 seconds, and then transferred to test plants for a feeding of approximately 1 hour.*

	Age of infection in days															
Leaf no.	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
I	4	3	5	3	4	0	5	4	5	5	5	8	6	2	6	
	Age of infection in days (cont.)															
Leaf no. cont.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
I	5	6	5	4[b]	4	5	6	3	1[c]	0[d]	1	1[e]	—	—	—	—
II	6	5	8	7	8	7	4	5	7	6	4	3	3	2[f]	0	2
III	4	6	6	8	6	4	6	6	5	7	4	6	7	1	2	2
IV	4	4	3	7	9	5	3	7	6	9	6	6	3	4	1	3
V	—	—	—	—	—	—	—	—	—	—	—	—	7	5	3	3
VI	—	—	—	—	—	—	—	—	—	—	—	—	4	4	5	5
VII	—	—	—	—	—	—	—	—	—	—	—	—	4	5	6	3

[a] Numbers in columns are plants infected out of 10 inoculated.
[b] Leaf showed necrotic veinbanding on basal portion.
[c] Leaf showed basal interveinal chlorosis.
[d] Leaf showed basal interveinal necrosis, petiole developed sunken areas.
[e] Three-fourths of leaf dried, recovery made from remaining flaccid tissue.
[f] Plant burned during nicotine fumigation.

flaccid tissue was successful. The following day the leaf and petiole had dried and recovery trials were discontinued.

After the plant had been infected 23 days recovery trials were begun from the 4 leaves which could be sampled readily. For practical purposes the leaves represented the oldest and the youngest leaves showing symptoms. Leaf I, approximately 8 mm. long, was the oldest; its symptoms have been described. Leaf II was the next oldest leaf; it was approximately 7.5 mm. long and showed a more expanded and diffused veinclearing symptom. Leaf III was the next to the youngest and was approximately 3.7 mm. long. This was the first leaf to show the mottle phase of the *Brassica nigra* virus disease. The majority of the blade area was pale green with scattered small, raised islands of normal green tissue. The youngest leaf that could be sampled, IV, was approximately 2 mm. long and had symptoms similar to those of III except that they were a lighter green.

When necrotic veinbanding symptoms were beginning on leaf I (26 days after inoculation) the approximate lengths of the 4 leaves were: I, 8.8 mm.; II, 8 mm.; III, 5 mm.; and IV, 3 mm. Recoveries were made from each leaf until, as noted previously, leaf I died (35 days after inoculation). At this time and continuing through the thirty-eighth day, recoveries were made from leaves II, III, and IV, and also from 3 additional younger leaves designated as V, VI, and VII. The approximate lengths of the 6 leaves at this time were: II, 8.5 mm.; III, 5.5 mm.; IV, 4.5 mm.; V, 5.0 mm.; VI, 3.7 mm.; and VII, 2.7 mm.

At the end of the thirty-sixth day the plant was injured by nicotine fumigation. Leaf II was severely burned and showed a green wilt symptom of nicotine injury the next day. Leaf III had approximately 0.6–0.12 mm of peripheral burn, while leaves IV and V showed only slight edge burning. This plant injury may have caused some variation in recovery trials from the most severely affected leaves (Table 4).

Inclusion of new leaves in recovery trial tended to compensate for loss of active virus in old leaves. Consequently the aggregate active virus in the plant would remain at a fairly high level for a relatively long time. This would be especially true when the measurement of active virus is not dependent on mass of plant tissue, but on the amount of active virus in tissues fed upon by aphids.

There have been various methods described for measuring the amount of virus present in plant extracts. These have been reviewed and appraised recently by both Takahashi (23) and Steere (18), and the advantages and disadvantages of the various methods have been pointed out. Some of the methods measure active virus (13, 14, 22), others measure more nearly the total virus nucleoprotein (2, 5, 22), while still others estimate particle numbers (1, 11, 18). The trials made in connection with the present work again demonstrate that insects can also be used to measure active virus present (6, 7, 9, 10, 16, 19, 24). This method has disadvantages, e.g., it involves the use of insects, it introduces another biological variable, and the procedure is somewhat tedious when done in proportions which would insure adequate samples of minimum variability. However, at least 3 advantages can be mentioned, 1) irreplaceable tissue is not lost for further assay, 2) it conceivably is applicable to situations where other methods have failed, and 3) it may

have application in cases where specific plant tissue areas are to be sampled, since insects vary in their sites of feeding. In the present work, the tissue area sampled was probably epidermis (4, 12).

SUMMARY AND CONCLUSIONS

Additional trials in transmission of the *Brassica nigra* virus by green peach aphids have indicated that some experimental variations can be traced to differences among plants, insect colonies, and virus sources. Fairly uniform results were obtained when 1 aphid colony, a single leaf virus source, were used to inoculate 1 group of uniform test plants on the same day within 2 hours.

Host plant susceptibility trials indicated that while aphids most frequently fed on the lower surface of seedling mustard, *Brassica juncea*, plants there was little difference in susceptibility of various tissue regions to infection. Placing seedlings in the dark for 24 or 48 hours prior to inoculation did not increase susceptibility when virus was introduced by insects under conditions of restricted feeding. There was no difference in susceptibility among plants varying from 1–5 weeks in age, as determined by insect inoculation. Differences among individual virus source plants existed, but there were no certain visible indications of such.

Using aphids, the course of active virus concentration was followed in single leaves of 5 plants for 15 days after symptom appearance, in 1 leaf of 1 plant from the time of symptom initiation until death (27 days), and in several leaves of 1 plant for 16 days. Apparently the ratio of active virus to amount of tissue was greatest some 15 days to 20 days following inoculation. In all leaves active virus concentration apparently went through a typical growth cycle. Active virus concentration, within the range and by the means tested, while at a low level in old leaves was at a fairly high level in younger leaves of the same plant. Mention is made of some of the advantages and disadvantages of using insects to determine relative active virus concentration in plants.

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TWO NEW SCAB-RESISTANT CUCUMBER VARIETIES [1]

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Cucumber (*Cucumis sativus* L.) is grown for pickle manufacture in Wisconsin to the extent of about 25,000 acres annually. Most of the acreage is located in central and northern counties. Scab (*Cladosporium cucumerinum* Ell. & Arth.) is regularly a major disease in the northern counties and is sometimes very destructive in the central counties. It is seldom found on the crop in the southern portion of the state in the latitude of Madison and to the south of that parallel. As already pointed out (4) the disease has a relatively low temperature optimum and consequently considerable variation in severity occurs from season to season in northern and central areas. The only scab-resistant pickle variety available has been Maine No. 2 developed at the Maine Experiment Station (1). Although this variety has been satisfactorily resistant in Wisconsin, certain horticultural characteristics limit its usefulness in this state. The most important of these is the long slender fruit which is not suitable for most manufacturing requirements. The second undesirable feature is the length of time from planting to first harvest which delays peak production to that time of the growing season when the frost hazard is great. The 2 varieties which have been in most common use are National Pickling and Chicago Pickling both of which have been described by Tapley, Enzie, and Van Eseltine (3). These have been supplemented in recent years, in areas where mosaic is important, by the mosaic-resistant varieties, Yorkstate Pickling (2) and Ohio MR 17 (6). None of the 4 varieties is resistant to scab. National Pickling was selected originally from Chicago Pickling and differs from the latter chiefly in that the fruits are less tapered toward the apex and the length of fruit is somewhat shorter in proportion to diameter.

After scab resistance was shown to be controlled by a single dominant gene (4), a breeding program was initiated with the objective of introducing resistance into Chicago Pickling and National Pickling. Two new scab-resistant varieties have now been perfected and released. Wisconsin SR 6 resembles National Pickling in type. Wisconsin SR 10 resembles Chicago Pickling. The purpose of the present paper is to record briefly the breeding methods used and the field performance of the lines from which the new varieties were chosen. A preliminary report has been published (5).

BREEDING PROCEDURE.—In a previous paper (4) it was shown that the resistant character could be evaluated best on young seedlings inoculated and held during the incubation period at about 17° C. In such environment plants homozygous for susceptibility were killed down to the cotyledonary node while those homozygous or heterozygous for resistance showed only minor injury and recovered promptly. This method was used throughout to evaluate breeding progenies for resistance.

The first cross, National Pickling × Maine No. 2, was carried to the F_3 generation to determine the nature of inheritance of resistance (4). Individuals from homozygous resistant F_3 progenies were then backcrossed to National Pickling. The first F_1 backcross progeny was completely resistant, as was expected. When F_1 individuals from the first backcross were again backcrossed to National Pickling, the second F_1 backcross progeny segregated into approximately equal numbers of resistant and susceptible plants. Resistant individuals of the second F_1 backcross were backcrossed to National Pickling and the third F_1 backcross segregated into approximately equal numbers of resistant and susceptible plants. Resistant individuals of the third F_1 backcross, when selfed, segregated into about 75 per cent resistant individuals. When certain of the latter group were selfed about ⅓ of the F_2 self progenies were homozygous for resistance. These homozygous resistant progenies showed fruit and vine type closely similar to National Pickling. Three progenies were increased for field evaluation and were designated as SR 1, SR 6, and SR 7.

Plants of the first F_1 backcross progeny of (National Pickling × Maine No. 2) × National Pickling were crossed with Chicago Pickling. Following the same procedure described in the preceding paragraph, 2 successive backcrosses to Chicago Pickling were made. From the second backcross, F_1 individuals were selfed and from the selfed progeny resistant individuals were again selfed. About ⅓ of the second self progenies were homozygous for resistance. They resembled rather closely fruit and vine type of Chicago Pickling. One of the progenies was increased for field study and designated as SR 10.

FIELD EVALUATION.—After greenhouse inoculation tests of seedlings of the mass increases of SR 1, SR 6, SR 7, and SR 10 showed them to be uniformly resistant, field trials were carried out to determine adaptability, yield, and fruit type, in comparison with standard varieties. The latter included National Pickling, Chicago Pickling, and Maine No. 2. At Green Bay and at Manawa, Wisconsin, in 1951, single 100-ft. rows of each variety and of SR 1, SR 6, and SR 7 were planted. A 33-ft. row of SR 10 was planted at Green Bay but yield records were not taken. At Madison, Wisconsin, in 1952, National Pickling, and SR 1, SR 6, SR 7, and SR 10 were included. Each lot tested was

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TABLE 1.—*Maturity and yields of scab-resistant lines and of standard varieties*

Variety or breeding line	Days to first pick			Yield in lbs. per acre		
	Manawa 1951	Green Bay 1951	Madison 1952	Manawa 1951	Green Bay 1951	Madison 1952
National Pickling	60	55	47	5670	4010	11200
Chicago Pickling	60	55	47	2730	3010	12800
Maine No. 2	67	62		5880	2800	
SR 1	57	55	47	6720	3990	11690
SR 6	57	55	47	7770	2870	13118
SR 7	57	55	47	7000	4130	11550
SR 10			49			9310
LSD 19:1						2030
LSD 99:1						2772

planted in 50-ft. rows in random order in each of 4 replicate blocks. Plots were picked at 3- to 4-day intervals according to the usual commercial procedures. At 1 or 2 pickings, random samples of fruits were measured as to length and diameter and the length/diameter ratio was determined.

In Table 1 are recorded the days from planting to first harvest and the total yields at each location. At the 2 locations where Maine No. 2 was included, the first picking of this variety was a week later than those of National Pickling and Chicago Pickling. At all 3 locations all the breeding lines were fairly close to National Pickling and Chicago Pickling in this regard. The percentage of diseased fruits approached 100 per cent in the susceptible varieties on August 24 at Green Bay and on August 30 at Manawa. Comparative yield records were necessarily discontinued at those dates. All resistant lines and Maine No. 2 remained free from visible foliage and fruit symptoms throughout the season. At Madison, scab did not appear on susceptible varieties and the yield records presented are for the entire season. In the 2 northern locations variation due to soil heterogeneity could not be analyzed and the comparative yields are only indicative in nature. At Madison where statistical analysis of the data was possible, there were no significant differences in yields of National Pickling, Chicago Pickling, SR 1, SR 6, and SR 7. SR 10, however, was significantly lower in yield than Chicago Pickling, SR 1, SR 6, and SR 7.

The ratios of fruit diameter to fruit length at the 3 locations are in Table 2. This characteristic varies somewhat with growing conditions and the ratio is consistently higher for each variety at Madison, where growing conditions were more favorable than at Manawa and Green Bay. Maine No. 2 had the slenderest fruits as shown by the largest value. At each location National Pickling was slightly shorter in relation to width than was Chicago Pickling. Of the 3 lines derived from backcrossing to National Pickling, SR 6 and SR 7 conformed closely with National Pickling. Fruits of SR 1 were consistently shorter in proportion to width than those of National Pickling. SR 10, which was derived from backcrossing with Chicago Pickling, had fruits which conformed rather closely with the latter variety in measurement.

Extensive commercial trials of SR 6, SR 7, and SR 10 in 1952 showed uniformly high resistance, good yielding capacity, satisfactory manufacturing quality, and reasonably good uniformity in plant and fruit characteristics. The color of fruits of SR 6 was slightly more typical of National Pickling than was that of SR 7. SR 7 was discarded on account of color and SR 1 was discarded because of its tendency to produce fruits that were too short. For practical purposes the SR 6 line, which has been released under the name Wisconsin SR 6, is a National Pickling type with the added characteristic of scab resistance. Likewise line SR 10, which has been released under the name Wisconsin SR 10, is a Chicago Pickling type with the added characteristic of scab resistance. Neither of these varieties carries resistance to mosaic. A breeding program directed toward incorporating the latter characteristic is under way.

SUMMARY

To meet the need for scab-resistant varieties of pickling cucumbers in northern and central Wisconsin a breeding program was initiated in which Maine No. 2 was used as the resistant parent in crosses with National Pickling and Chicago Pickling, the standard varieties in general use. After an initial cross between Maine No. 2 and National Pickling and the isolation of homozygous F_3 progenies, 3 successive backcrosses to National Pickling were made. Plants of the first backcross were crossed with Chicago Pickling and backcrossed twice to the latter variety. Resistant plants

TABLE 2.—*Length/diameter fruit ratios of scab resistant lines and standard varieties*

Variety or breeding line	Length/diameter ratio at			
	Manawa 1951	Green Bay 1951	Madison 1952	Average
National Pickling	2.80	2.68	2.91	2.80
Chicago Pickling	2.87	2.82	3.22	2.97
Maine No. 2	3.30	3.53		3.42
SR 1	2.58	2.63	2.75	2.65
SR 6	2.74	2.85	2.99	2.86
SR 7	2.68	2.71	3.01	2.80
SR 10		2.98	3.06	3.02

in each segregating progeny were selected after inoculation in the seedling stage under optimum conditions for disease development. From each backcross program homozygous resistant progenies were evaluated in field trials for time of maturity, yielding capacity, fruit shape and color, and pickling quality. From this study 2 new varieties were selected, increased, and released. Wisconsin SR 6 is similar to National Pickling in horticultural characteristics and has the additional quality of resistance to scab. Wisconsin SR 10 resembles Chicago Pickling in type and is also homozygous for resistance to scab.

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PHYTOPATHOLOGICAL NOTES

Potato Virus Y in Hawaii[1]. K. SAKIMURA. A mixed natural infection of potato virus Y, *Marmor upsilon* (H.), and spotted wilt virus, *Lethum australiense* H., was discovered among field plantings of tomato (*Lycopersicon esculentum* Mill.). The former was isolated after subinoculation on *Nicotiana glutinosa* L., and was studied for physical properties, host range, symptoms on various hosts, and vector species. In Hawaii, Parris[2,3,4] first recognized the virus through the symptoms on potato and possibly on tomato as early as 1937. The present study confirms his determination and establishes for the first time its identity through experimental evidence. Some observations were made on field incidence of the disease in 1950 and 1951.

The thermal inactivation point observed was between 53 ° and 56° C with 10 minutes exposure time. The longevity *in vitro* observed was between 48 hours and 72 hours at 25–29° C, 66 days and 82 days at 0–3° C. The dilution end point observed was between 1:300 and 1:1,000. These test inoculations were all made on *N. glutinosa*. The findings are fully in accord with those recorded for potato virus Y.

The virus was experimentally transmitted through juice inoculation to *Solanum melongena* L., *S. nodiflorum* Jacq., *Nicotiana glutinosa* L., *N. rustica* L., *N. tabacum* L., *Physalis peruviana* L., *Capsicum frutescens* L., *Petunia hybrida* Vilm., *Nicandra physaloides* (L.), and *Lycopersicon esculentum* Mill. The symptoms observed on these hosts were identical with those described for potato virus Y. Several variations were observed in severity and type of symptoms on various host plants among the isolates derived from many field infected samples. Two varieties of *S. melongena*, Japanese Long and New York Improved, were observed, in repeated tests, to be symptomless but locally infected by the isolates used for inoculation. *P. peruviana* did not show any local lesions such as observed on *P. floridana* Rydb.,[5] at the temperature range of 12–29° C, but showed conspicuous systemic symptoms. *Ananas comosus* Merr., *Emilia sonchifolia* DC. and *Datura stramonium* var. *chalybea* Koch, in inoculation and recovery tests, were observed to be immune from the virus.

The virus was readily transmited to *N. rustica* test plants by *Myzus persicae* (Sulzer) after a 1–2-day acquisition feeding and a 3–4-day infection feeding of 5–15 apterous young aphids per plant.

Observations made in a truck-farming district on the island of Oahu revealed that field plantings of tomato were commonly infected with potato virus Y throughout the district. Many field sample materials were processed through subinoculation tests. Incidence on tomato was also observed in several other localities. Natural infection of peppers was also observed. As potatoes are rarely grown on the island of Oahu, there has been no opportunity to make observations on this host.

S. nodiflorum is one of the most common weeds growing throughout the irrigated and non-irrigated lowland cultivated areas. Infection of this weed host, often common in localized spots, was observed to be

[1] Published with the approval of the Director of the Pineapple Research Institute of Hawaii as Technical Paper No. 208.
[2] Parris, G. K. 1938. Potatoes. Rept. Hawaii Agr. Exp. Sta. for 1937. 39-40.
[3] Parris, G. K. 1938. The diseases of truck crops in Hawaii. Univ. Hawaii Agr. Ext. Serv. Ext. Bul. 33: 1-78.
[4] Parris, G. K. 1940. A check list of fungi, bacteria, nematodes, and viruses occurring in Hawaii, and their hosts. U. S. Dept. Agr. Plant Dis. Reptr. Suppl. 121: 1-91.
[5] Ross, A. F. 1948. Local lesions with potato virus Y. Phytopathology 38: 930-932.

widely scattered over the various habitats, such as truck farms, pineapple and sugar cane fields, roadsides, and home gardens in the city. It was particularly common in the truck-farming areas. Scoutings were made extensively over the island of Oahu and many field sample materials were processed through subinoculation tests. Field symptoms of the disease are inconspicuous and indefinite, particularly on older leaves or on plants with older infection, which often remain nearly symptomless.

S. nodiflorum, under subtropical surroundings in Hawaii, grows continuously without interruption in its succession throughout the whole year, and often a semi-shrubby old growth is present in neglected areas. Its abundance, uninterrupted succession, long life, and high incidence of infection clearly suggest that this weed should be the major reservoir plant of the virus under natural conditions. Infestation of aphids on this host plant was occasionally observed in the fields.

This virus must have been introduced to Hawaii in potato tubers. Potatoes have been continuously imported from the Pacific Coast states during the past 100 years, mostly for food but partly for planting purposes. Although the disease was first noted in 1937, its introduction to Hawaii could have occurred many years before that date.—Pineapple Research Institute of Hawaii.

Rosette of Rose. H. Earl Thomas and C. Emlen Scott In midsummer of 1941, a clinical specimen of *Rosa rubrifolia* was received through the kindness of Dr. G. Weston Bohn from Mr. G. M. Willson, Superintendent, State Training School, Lander, Wyoming, where it was grown as an ornamental shrub. What appeared to be the same disease was found by Mr. Willson in 1942 in an unidentified native rose near the same site. The junior author collected the same or a similar disease in a native rose, possibly *R. pisocarpa*, near Carrville in a mountainous area of Trinity County, California, at about the same time. The relation of these collections to the disease or diseases reported in Manitoba as witches' broom[1] and later as mosaic[2] has not been determined.

The disease was characterized by misshapen leaflets and flower parts if present, dwarfing of stems, precocious growth of lateral buds, an indefinite chlorotic pattern in leaves and an increase in thorniness on affected stems of *R. rubrifolia*.

Since the disease appeared to be destructive to the roses in which it was found it was important to determine its effect on some of the roses commonly used as rootstocks in the Western United States and in some of the native roses. This has not been fully accomplished but some facts can be reported now. The disease from each source was readily, though slowly, transmitted to healthy rose plants by grafting, indicating, as did the symptoms, that a virus is the cause. The results, barring minor differences, indicate that the 2 collections represent a single disease.

Fig. 1. Stem and leaves of *R. odorata* affected by the Wyoming strain of rosette virus, with a normal leaf of the same species.

The Wyoming virus was inoculated by grafting into *Rosa californica*, *R. multiflora*, *R. nutkana*, *R. odorata*, *R. rubrifolia*, *R. spinosissima*, Belle of Portugal, and Ragged Robin. All but *R. californica* and *R. spinosissima* developed strong symptoms in 3–14 months in at least some parts of the plants. An extreme case is shown in Fig. 1. The 2 last named species had no symptoms at all, although *R. Californica* was kept under observation 17 months. No clear increase in thorniness was noted in any kind except *R. rubrifolia*. There was an increase in red pigment in leaves of infected Ragged Robin. All affected plants eventually died, perhaps partly from secondary causes. A single attempt to infect *Holodiscus discolor* with this virus apparently failed.

The following roses were inoculated with the California virus: *R. californica*, *R. multiflora*, *R. nutkana*, *R. odorata*, *R. pisocarpa* and Ragged Robin. The first, third, and fifth species are native to northern California. There were no symptoms on *R. californica* after 42 months, nor on *R. pisocarpa* after 26 months, nor on *R. multiflora* after 23 months. Strong symptoms appeared on *R. nutkana* near the point of inoculation after 19 months. Symptoms were apparent on *R. odorata* after 19 months but not at 14 months. In 1 case, Ragged Robin was nearly but not fully invaded in 17 months. Attempts to infect the native *Fragaria chiloensis* and *Prunus ilicifolia* failed to produce symptoms. The California virus seemed on the whole slower than the Wyoming in development in the plants tested, but since the 2 diseases appear to be otherwise identical, the viruses are tentatively regarded as the same.

While this virus disease is capable of severe damage

[1] Conners, I. L. 1941. Twentieth Ann. Rep. Canad. Plant Dis. Survey. 1940: 104 p. (p. 98).

[2] Conners, I. L. 1942. Twenty-first Ann. Rep. Canad. Plant Dis. Survey. 1941: 102 p. (p. 98).

to several of the species and varieties of roses commonly grown, it is thus far only a potential threat to rose culture in California. The slow rate of development of the disease coupled with the experience to date in the Wyoming planting indicates that eradication of the disease will be possible but not easy. A few affected plants were still to be found at Lander in 1945.—Department of Plant Pathology, University of California, Berkeley, California.

Spore Germination in Tilletia asperifolia, a Species Unique to the Genus. C. S. HOLTON AND W. N. SIANG.[1] For taxonomic purposes an adequate description of any species belonging to the genus *Tilletia* should include a description of spore germination. Unfortunately, such information is either wanting or vague in most taxonomic treatments of this genus and its specific components. This was indicated by Clinton[3] who stated that the spores of many of the species had never been germinated but the species had been placed in the genus by spore characteristics only. Spore germination was reported for only 4 of the 19 species described by Clinton[3] in 1904. Two of the 4 species, the spores of which germinated, were *Tilletia caries* and *T. foetida*. These same 2 species were among 6 for which spore germination was described by Fischer and Hirschhorn[4] in 1945.

Since germination is difficult to induce in chlamydospores of many *Tilletia* species, the current concept of spore germination for this genus proably is based on the more commonly encountered *T. caries* and *T. foetida*. According to Clinton[3], germination in *Tilletia* usually is "by a short promycelium which bears a terminal cluster of elongated sporidia that, with or without fusing in pairs, may in nutrient solution give rise to a considerable mycelium bearing secondary air-sporidia." This description becomes increasingly inadequate as new methods of inducing spore germination make possible observations on a wide range of species. An example in point is *Tilletia asperifolia* Ell. & Ev., on *Muhlenbergia asperifolia* (Nees & Mey.) Parodi[4]. Recent observations on spore germination in this species indicate that it is unique to *Tilletia*, particularly with reference to the morphology and behavior of the primary sporidia.

Chlamydospores of *T. asperifolia* were incubated on 2 per cent water agar at 15°–18° C. and at 8° C. Periodic observations showed that germination occurred in 1 week at the higher temperature and in 2 weeks at the lower temperature. Germination was estimated to be above 50 per cent in all tests. Each spore germinates by the production of a promycelium which bears a whorl of primary sporidia at the apex. These primary sporidia are in distinct contrast to those of other species of this genus that have been described. They are relatively short (20–30μ.), broadly cylindric, and rounded at the tip (Fig. 1, A, B). The number of primary sporidia on the promycelium may range from 5 to 15. The average number observed on 60 promycelia was 8.9, the most frequent numbers being 8 and 9. The primary sporidia fuse in pairs, and the union always occurs at or near the base (Fig. 1, C). Both single and fused primary sporidia give rise to sickle-shaped secondary sporidia (Fig. 1, D) which are forcibly discharged from the sterigmata. The production, discharge, and development of secondary sporidia is the same as in *T. caries*.

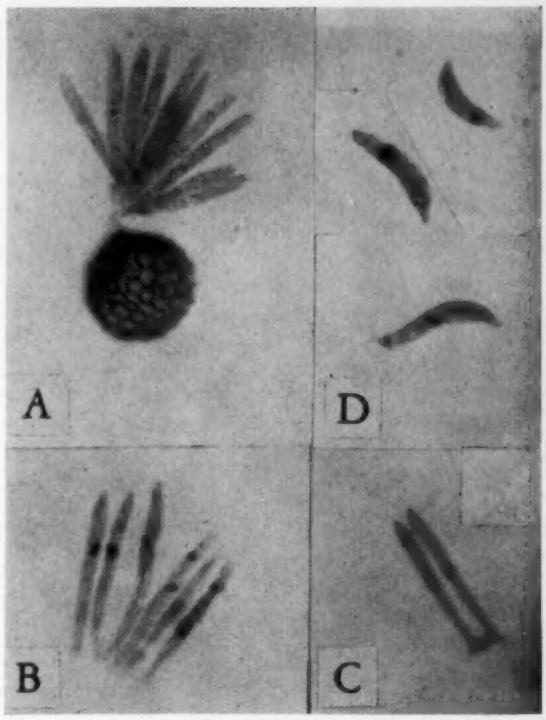

FIG. 1. Photomicrograph of stained preparation of *Tilletia asperifolia* illustrating spore forms and nuclear behavior. A) Germinated chlamydospore bearing 10 primary sporidia. ×780. B) Four uninucleate and 2 binucleate primary sporidia from 1 promycelium ×1300. C) A pair of fused primary sporidia. ×780. D) Three uninucleate secondary sporidia. ×1300.

Tilletia asperifolia grows readily on nutrient media. Cultures may be obtained by isolating single primary sporidia or fused pairs of primary sporidia. Both single and fused pairs of primary sporidia produce mycelial colonies which give rise to sickle-shaped

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. Agriculture, and the Washington Agricultural Experiment Station. Scientific Paper No. 1166, Washington Agricultural Experiment Stations, Pullman.

[2] Pathologist, U. S. Dept. of Agriculture and Research Assistant, Wash. Agr. Exp. Sta., Pullman, Wash., respectively.

[3] Clinton, G. P. 1904. North American Ustilagineae. Proc. Boston Soc. Nat. History. 31: 329-529.

[4] Fischer, G. W., and Elisa Hirschhorn. 1945. The Ustilaginales or "Smuts" of Washington. Wash. Agr. Exp. Sta. Bul. 459. 84 pp.

secondary sporidia. Growth of fused pairs is more rapid in the early stages than that of single sporidia, indicating a stimulating influence of nuclear association in the dikaryophase. This differential rate of growth gradually disappears, suggesting a reversion to the haplophase. Presumably, therefore, colonies originating from fused pairs of primary sporidia are composed of a mixture of sexually opposite haploid components. This apparent capacity to revert from the dikaryophase to the haplophase in culture has not been reported for other *Tilletia* species.

Primary and secondary sporidia were stained with Heidenhain's iron haematoxylin. Both kinds of sporidia are uninucleate, although an occasional binucleate one may be seen (Fig. 1 B, D). The nuclear condition of chlamydospores and the early stages of nuclear division have not been determined.

The distinctive characteristics of *T. asperifolia* reported here may have significance in relation to phylogeny. While the chalmydospores are characteristic of *Tilletia*, the primary sporidia resemble those of *Entyloma* and *Urocystis*. There is also some resemblance to *Tilletia euphorbia*.[5]—Washington Agricultural Experiment Stations, Pullman, Washington.

[5] Lenz, L. Wayne. 1941. A new smut from Louisiana. Mycologia. 33: 155-157.

Survival of Colletotrichum gossypii on Cotton Seeds in Storage. C. H. ARNDT. In a previous paper[1] data are given on the survival of *Colletotrichum gossypii* South. infesting cotton seed of the varieties Carolinadel and Deltapine after storage for 5.5 years at 4 temperatures and at moisture contents of 8, 10, 12, 14, and 16 per cent.

When seed from the same lots were germinated by Simpson[2] after 13.5 years of storage, there were no viable seeds in the sub-lots stored at the fluctuating temperature of a storage house and at 33°C. At 21°C., the only viable seeds were those in the 2 sub-lots of 8 per cent moisture content; while the sub-lots stored at 1°C. and at moisture contents of 14 per cent or less still showed at least 70 per cent viable seeds.

Portions of the viable sub-lots were forwarded to the writer, who germinated the seeds by the method previously described[1], to ascertain the percentage of seedling emergence, the number of seedlings infected by fungi, and the species of the infecting fungi. The data reported in Table 1 were derived from the culture of 100 seeds of each sub-lot at 24°C. for 12 days. The only sub-lot after the 13.5-year storage period in which a large percentage of seedlings were infected by the anthracnose fungus was the Deltapine sub-lot of 8 per cent moisture, stored at 1°C. A small percentage of the seedlings of 2 other sub-lots stored at 1°C., Carolinadel of 10 per cent and Deltapine of 12 per cent moisture content, were also infected by the anthracnose fungus. An anomaly appears in the failure of the fungus to survive at 1°C. on the Carolinadel sub-lot of 8 per cent moisture content. *Rhizopus nigricans* Ehr. infected and destroyed a few cotyledons in 4 sub-lots. *Fusarium moniliforme* Shel. was identified on about 10 per cent of the seedlings infected by *C. gossypii* and also on several other seedlings, which seems to indicate that it also survived on the seedlings during the storage period.—South Carolina Agricultural Experiment Station, Clemson, S. C.

[1] ARNDT, C. H. 1946. Effect of storage conditions of the survival Colletotrichum gossypii. Phytopathology 36: 24-29.

[2] Personal communication. Mr. D. M. Simpson.

TABLE 1.—*Effect of storage for 13½ years on germination of 2 lots of cotton seed, percentage of seedlings infected, and fungi involved*

Storage temperature	Moisture content	Carolinadel No. 2			Deltapine 11a		
		Emergence	Infected seedlings	Infecting fungi	Emergence	Infected seedlings	Infecting fungi
	Per cent	Per cent	Per cent		Per cent	Per cent	
1°C	8	95	1	Fm[a]	93	70	Cg, Fm
	10	88	12	Cg, Fm	90	5	Rn, Fm
	12	75	5	Rn, Fm	90	5	Cg
	14	73	8	Rn	70	2	Rn
	16	3	0	0	5	0	0
21°C	8	75	2	Fsp.	28	0	0

[a] Abbreviations for infecting fungi are: Cg, *C. gossypii;* Fm, *Fusarium moniliforme;* Fsp, *Fusarium* spp. other than *F. moniliforme;* Rn, *Rhizopus nigricans.*